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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/34, C07D 307/83, 317/44, A61K 31/36, A61K 31/44, 31/47, 31/535, C07D 215/38, C07D 317/70, 319/12, 401/02, 413/10 — A1

(11) International Publication Number: WO 90/09788

(43) International Publication Date: 7 September 1990 (07.09.90)

(21) International Application Number: PCT/US90/00842

(22) International Filing Date: 23 February 1990 (23.02.90)

(30) Priority data:
313,826 — 23 February 1989 (23.02.89) — US
406,330 — 12 September 1989 (12.09.89) — US

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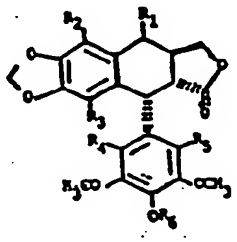
(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

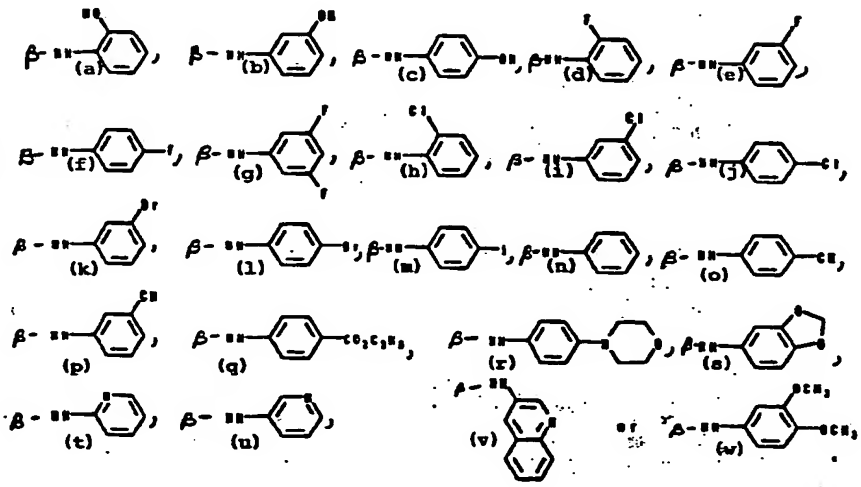
(54) Title: ETOPOSIDE ANALOGUES

(57) Abstract

Compounds that are analogs of etoposide and exhibit antitumor activity are disclosed. The compounds of the present invention have formula (I), where R_1 is β-$OCH_2CH_2NH_2$, β-$NHCH(CH_3)CH_2OH$, β-$NHCH_2CH(CH_3)OH$, β-Cl, β-Br, β-OH, α-OH, β-NH_2, α-NH_2, β-$NHCH_2CH_2OH$, α-$NHCH_2CH_2OH$, β-$NHCH_2CH_2CH_3$, β-$NHCH_2CH_2OCH_3$, β-$NHCH_2CH=CH_2$, β-$NHCH_2CH(OH)CH_3$, β-$NHCH_2CH_2CH_2OH$, β-OCH_2CH_2OH, (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m), (n), (o), (p), (q), (r), (s), (t), (u), (v) or (w). R_2 is H, or Br; R_3 is H, or Br; R_4 is H, or Br; R_5 is H, or Br; and R_6 is H, or $-CH_3$.



(I)



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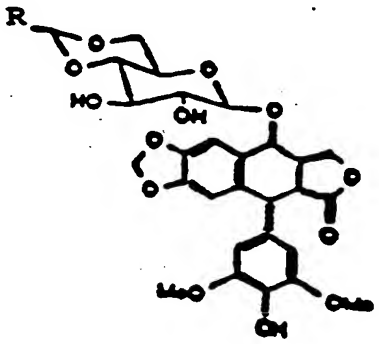
ETOPOSIDE ANALOGUES

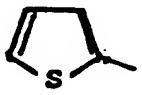
This invention relates to compounds that are analogs of etoposide having antitumor activity. This invention also relates to a method for treating tumors by administering a safe and effective amount of the etoposide analog compounds.

BACKGROUND OF THE INVENTION

Podophyllotoxin is a naturally occurring compound extracted from the mandrake plant. Recently two therapeutically useful semi-synthetic glycosides of podopyllotoxin, etoposide (also known as VP-16), shown below, and teniposide (also known as VM-26), have been developed.



R=CH_3 (Etoposide)

R=  (Teniposide)

These compounds exhibit therapeutic activity in several human neoplasms, including small cell carcinomas of the lung, testicular tumors, Hodgkin's disease, Papillomavirus, and diffuse histiocytic lymphoma.

It is believed that these drugs block the catalytic activity of DNA topoisomerase II by stabilizing an enzyme-DNA complex in which the DNA is cleaved and covalently linked to the enzyme. See Chen, G. L., Yang, L., Rowe T. C., Halligan, B.D., Tewey, K., and Liu, L., J. Biol.

Chem., 259, 13560 (1984); Ross, W., Rowe, T., Glisson, B., Yalowich, J., and Liu, L., Cancer Res., 44, 5857 (1984); Rowe, T., Kuppfer, G., and Ross, W., Biochem. Pharmacol., 34, 2483 (1985), which are all herein specifically incorporated by reference. By way of background, topoisomerases are enzymes which control the topological state of DNA. Type II topoisomerases catalyze DNA strand passage through transient double strand breaks in the DNA. The resulting change in the linking number of DNA allows these enzymes to mediate DNA interconversions, such as supercoiling and relaxation of supercoiling, catenation and decatenation, knotting, and unknotting. See Wang, J. C., Annu. Rev. Biochem., 54, 665 (1985) and Maxwell, A., and Gellert, M., Adv. Protein Chem., 38, 69 (1986), which are herein specifically incorporated by reference.

Type II DNA topoisomerase enzymes have been shown to be involved in a number of vital cellular processes, including DNA replication and transcription, and chromosomal segregation. These enzymes, therefore, are a critical target for the action of a wide variety of anticancer drugs, including etoposide and teniposide. The key step leading to cell death may be the capability of these drugs to block the catalytic activity of DNA topoisomerase II, as noted above.

Structure-activity studies have demonstrated a direct correlation between cytotoxicity, DNA breakage, and murine-derived topoisomerase II inhibition activities among the podophyllotoxin analogues. See Minocha, A., and Long, B., Biochem Res. Comm., 122, 165 (1984), which is herein specifically incorporated by

reference. The isolation and purification of human type II topoisomerase from lymphocytic leukemia cells has provided the means to use this enzyme as a target to investigate the structure-activity relationships among etoposide and related congeners.

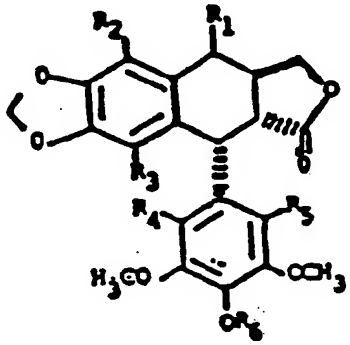
It has been shown that the substitution of etoposide's glycosidic moiety by an 4-alkoxy group, as in 4'-demethyl-epipodophyllotoxin ethyl ether, preserves the inhibitory activity of DNA topoisomerase II intact at higher concentrations. See Thurston, L.S., Irie, H., Tani, S., Han, F. S., Liu, Z. C., Cheng, Y.C., and Lee, K. H., J. Med. Chem., 29, 1547 (1986), which is herein specifically incorporated by reference. However, it has also been shown that a series of 4-acyl congeners are less active, even though some of them possessed potent cytotoxicity. See Thurston, L. S., Imakura, Y., Haruna, M., Li, D. H., Liu, Z. C., Liu, S. Y., Cheng, Y. C., and Lee, K. H., J. Med. Chem., 31, (1988), which is herein specifically incorporated by reference.

Summary of the Invention

The present invention provides novel compounds which exhibit antitumor activity. The compounds are analogs of etoposide. More specifically, preferred compounds of the present invention are etoposide analogs wherein the glycosidic moiety is replaced by various substituents, such as a 2"-hydroxyethylamino chain, a 2"-methoxyethylamino chain, a 4''-fluoroanilinyl chain, a chlorine atom, or a bromine atom.

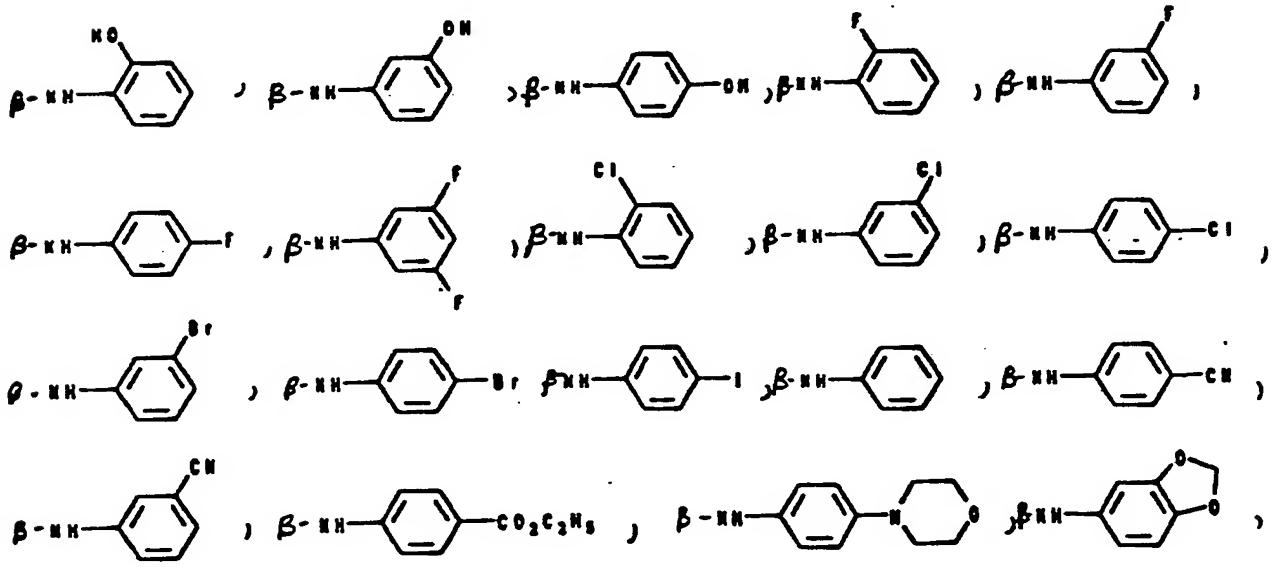
present invention. The compounds of the present invention have been shown to inhibit type II human topoisomerase and also to cause cellular protein-linked DNA breakage and, therefore, may be useful in the treatment of tumors. The compounds may also be useful in the treatment of papilloma virus.

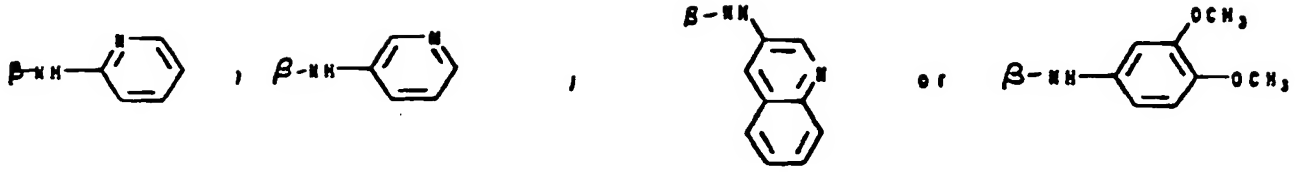
In accordance with the present invention, there are provided compounds of the formula:



wherein

R_1 is β-$OCH_2CH_2NH_2$, β-$NHCH(CH_3)CH_2OH$, β-$NHCH_2CH(CH_3)OH$, β-Cl, β-Br, β-OH, α-OH, β-NH_2, α-NH_2, β-$NHCH_2CH_2OH$, α-$NHCH_2CH_2OH$, β-$NHCH_2CH_2CH_3$, β-$NHCH_2CH_2OCH_3$, β-$NHCH_2CH{=}CH_2$, β-$NHCH_2CH(OH)CH_3$, β-$NHCH_2CH_2CH_2OH$, β-OCH_2CH_2OH,





R_2 is H, or Br;

R_3 is H, or Br;

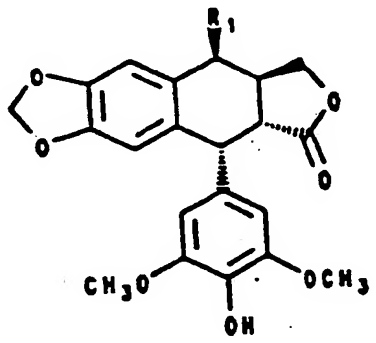
R_4 is H, or Br;

R_5 is H, or Br; and

R_6 is H, or $-CH_3$.

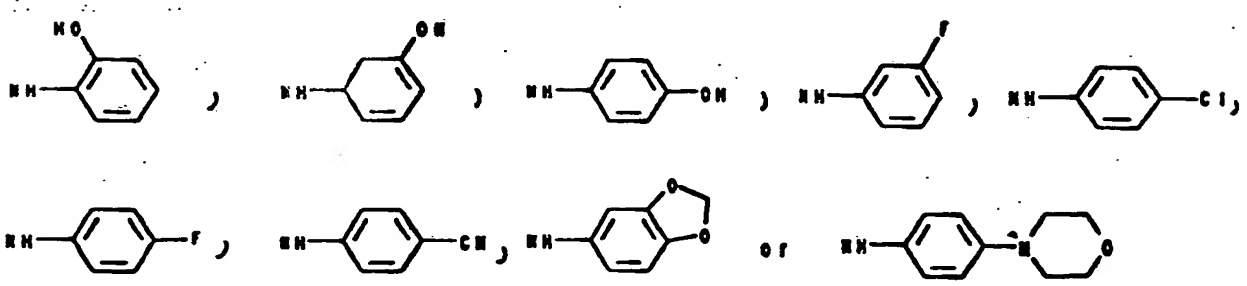
The present invention is also for a process for treating tumors in humans and lower animals by administering a safe and effective amount of a compound as described above.

A preferred group of compounds within the present invention exhibit inhibitory activity on human type II DNA topoisomerase to an equal or greater extent than etoposide and are of the formula:



wherein

R_1 is $-NHCH_2CH_2OH$, $-NHCH_2CH_2OCH_3$, $NHCH_2CH(OH)CH_3$, $NHCH(CH_3)CH_2OH$, Cl, $OCH_2CH_2NH_2$.



Particularly preferred compounds include 4'-Demethyl-4ß-amino-4-desoxypodophyllotoxin; 4'-Demethyl-4ß-[2"-hydroxyethylamino]-4-desoxy-podophyllotoxin; 4'-Demethyl-4ß-[2"-hydroxypropylamino]-4-desoxy-podophyllotoxin; 4'-Demethyl-4ß-[1"-methyl-2"-hydroxyethylamino]-4-desoxypodophyllotoxin; 4ß-Chloro-4-desoxy-podophyllotoxin; 4'-demethyl-4ß-Chloro-4-desoxypodophyllotoxin; 4'-Demethyl-4ß-[3"-hydroxyanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4ß-[2"-hydroxyanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4ß-[4"-hydroxyanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4β-[2"-fluoroanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4β-[3"-fluoroanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4β-[4"-fluoroanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4β-[3",5"-difluoroanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4β-[4"-chloroanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4β-[4"-bromoanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4β-anilinyl-4-desoxypodophyllotoxin; 4'-Demethyl-4β-[4"-cyanoanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4β-[3"-cyanoanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4β-[4"-ethoxycarbonylanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4β-[4"-morpholinoanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4β-[3",4"-methylenedioxyanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4β-[3",4"-dimethoxyanilinyl]-4-desoxypodophyllotoxin; 4'-Demethyl-4β-[3"-pyridylamino]-4-

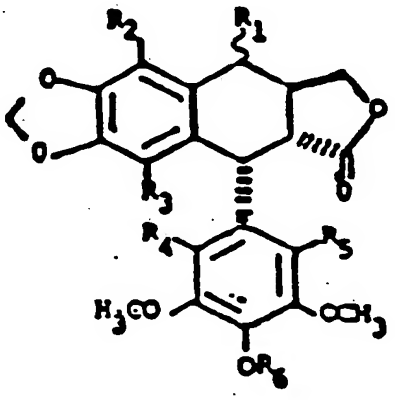
desoxypodophyllotoxin; and 4'-Demethyl-4β-[3"-quinolinylamino]-4-desoxypodophyllotoxin.

Additional objects and advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned from the practice of the invention.

Description of The Preferred Embodiments

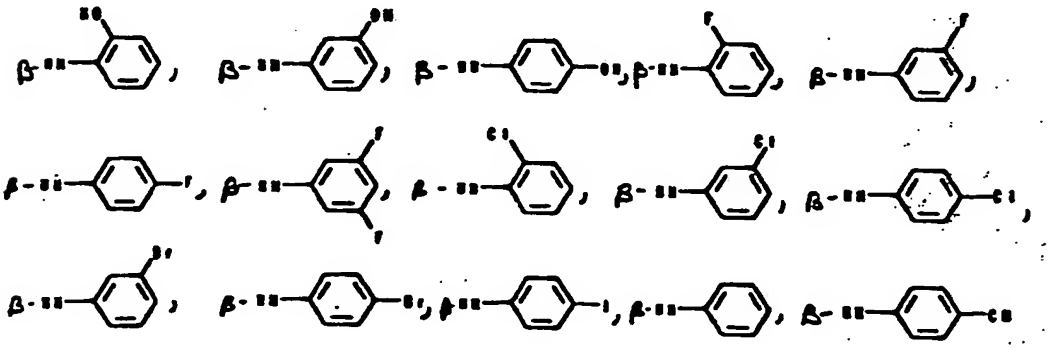
Reference will now be made in detail to the presently preferred embodiments of the invention, which together with the following examples, serve to explain the principles of the invention.

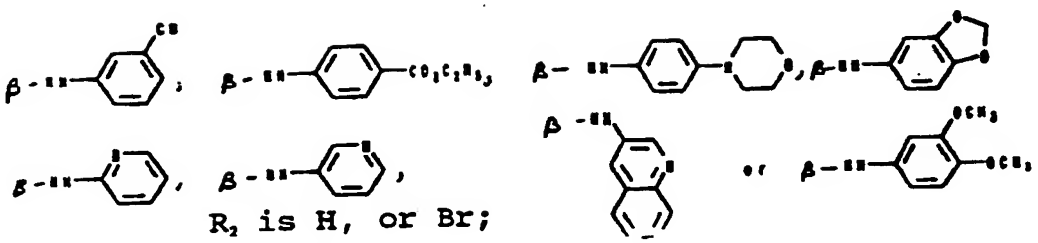
As noted above, the present invention relates to compounds of the formula:



wherein

R_1 is β-$OCH_2CH_2NH_2$, β-$NHCH(CH_3)CH_2OH$, β-$NHCH_2CH(CH_3)OH$, β-Cl, β-Br, β-OH, α-OH, β-NH_2, α-NH_2, α-$NHCH_2CH_2OH$, β-$NHCH_2CH_2CH_3$, β-$NHCH_2CH_2OCH_3$, β-$NHCH_2CH{=}CH_2$, β-$NHCH_2CH(OH)CH_3$, β-$NHCH_2CH_2CH_2OH$, or -OCH_2CH_2OH,





R_2 is H, or Br;

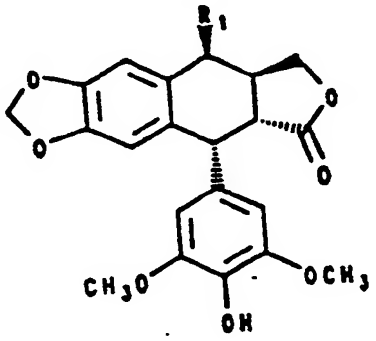
R_3 is H, or Br;

R_4 is H, or Br;

R_5 is H, or Br; and

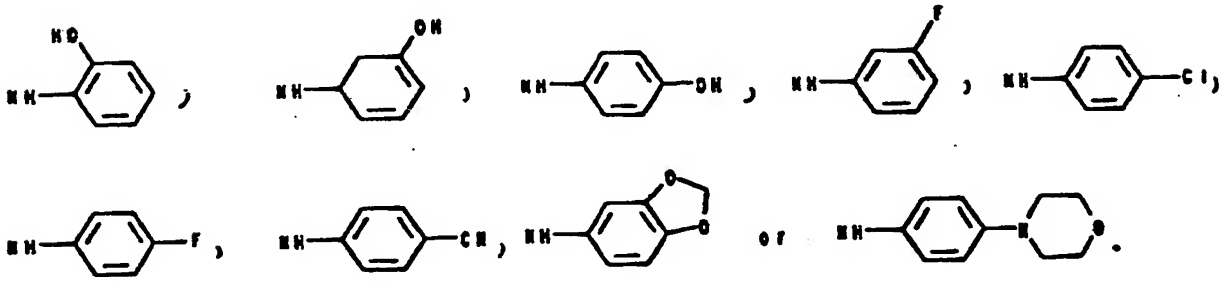
R_6 is H, or $-CH_3$.

As noted above, a preferred group of compounds within the present invention exhibit inhibitory activity on human type II DNA topoisomerase to an equal or greater extent as etoposide and are of the formula:

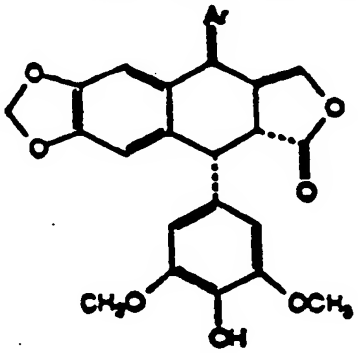


wherein

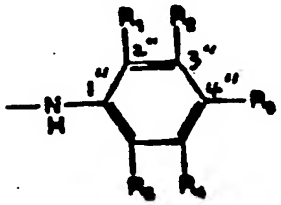
R_1 is $NHCH_2CH_2OH$, $NHCH_2CH_2OCH_3$, $NHCH_2CH(OH)CH_3$, $NHCH(CH_3)CH_2OH$, Cl, $OCH_2CH_2NH_2$,



Another preferred group of compounds under the present invention will possess a 4'-demethyl-4β-substituted anilinyl 4-desoxypodophyllotoxin wherein the compound possesses the following formula.

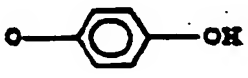


wherein Ar is an arylamine as in the formula below,



wherein R_1 is H, OH, F, Cl, Br, CO_2CH_3, $CO_2C_2H_5$, CN, NO_2, NH_2, $N(CH_3)_2$, OCH_3, CH_2OH, CH_3, CF_3, CH_2CH_2OH, $COCH_3$, CH_2NH_2;

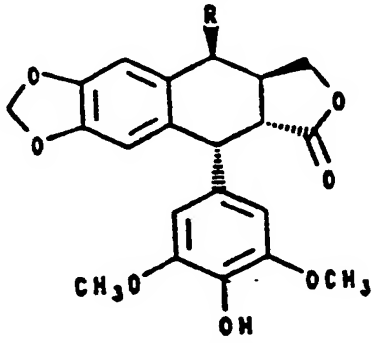
R_2 is H, OH, F, Cl, Br, CO_2CH_3, $CO_2C_2H_5$, CN, NO_2, NH_2, $N(CH_3)_2$, OCH_3, CH_2OH, CH_3, CF_3, CH_2CH_2OH, $COCH_3$, CH_2NH_2, $CHOHCH_3$, SCH_3, CH_3, CO_2CH_3;

R_3 is H, OH, F, Cl, Br, I, CO_2CH_3, $CO_2C_2H_5$, CN, NO_2, NH_2, $N(CH_3)_2$, OCH_3, CH_2OH, CH_3, CF_3, CH_2CH_2OH, $COCH_3$, CH_2NH_2,  ,  ,  , $N(CH_2CH_2OH)_2$, CH_3;

R_4 is H, F, Cl, OH, OCH_3, CO_2CH_3, $CO_2C_2H_5$, CH_3, CF_3, NO_2, NH_2, Cl;

R_5 is H, F, Cl, CH_3, CF_3, OH, OCH_3, NO_2;
R_3 and R_4 are OCH_2O or OCH_2CH_2O.

The preferred compounds of the present invention can also be more generally described as a compound of the formula:



wherein R is a flat aromatic group which may contain a heteroatom or alternatively may contain electron donating substituents at the 3" or 4" position of the aromatic ring. Specifically the substituents may be oxygen containing groups, or the aromatic group may be pyridine.

The compounds of the present invention are analogs of etoposide wherein the glycosidic moiety has been replaced. Compounds exhibiting potent inhibition of human DNA topoisomerase II result from replacing the glycosidic moiety with a 2"-hydroxyethylamino chain, a 2"-methoxyethylamino chain, or a substituted arylamine at the C-4ß position. Inhibitory activity can also be increased by substituting the glycosidic moiety by chlorine, bromine, or an amino group at the C-4ß position. It is believed that the stereochemistry of the 4ß-substituents plays an important role in

determining inhibitory potency. In general, β-isomers exhibit greater activity than the corresponding α-isomers. Another factor affecting the potency is the length of the substituent group at the C-4 position and the substitution on that group. This distance factor is different for halide and hydroxy substituted arylamines. For the hydroxy substituted arylamine, the meta position showed the most potency, while in the halogens, para substitution showed the most potency. A second potency factor regarding the halogen substituted aryl amines is the size of the halogen. Fluorine, the smallest has the most potency, while Iodine, the largest has the least. In addition, substitution of bromine at either one or more of the R_2, R_3, R_4, and R_5 positions will result in compounds having significant inhibitory activity. R_6 can be varied by substituting hydrogen with a methyl group. These modifications will produce changes in inhibitory activity which can be readily determined by assays known in the prior art through the exercise of routine skill in light of the teachings contained herein.

The compounds of the present invention were tested for their degree of inhibitory activity on human type II DNA topoisomerase, their effect on the formation of protein-linked DNA breakage, and their cytotoxicity. The inhibitory activity for compounds of the present invention correlated with the ability of the compounds to cause DNA strand breakage. However, the <u>in vitro</u> cytotoxicity of the compounds tested did not appear to correlate with the enzyme inhibitory activity and DNA strand break activity. The results of the tests

on some of the compounds of the present invention are shown in Tables I and III. For a description of the assays used with respect to the compounds listed in Tables I and III see Thurston, L.S., Irie, H., Tani, S., Han, F. S., Liu, Z. C., Cheng, Y.C., and Lee, K. H., Antitumor Agents 78. Inhibition of Human DNA Topoisomerase II by Podophyllotoxin and α-Peltatin Analogues, J. Med. Chem. 29, 1547 (1986), and the references cited therein.

Tables I, and III illustrate the inhibitory activity, DNA strand breakage ability, as well as the cytotoxicity of etoposide and some of the compounds of the present invention. As shown in Tables I and III, the inhibitory activity of 4'-Demethyl-4ß-amino-4-desoxypodophyllotoxin (Example 3), 4'-Demethyl-4ß-[2"-hydroxyethylamino]-4-desoxy-podophyllotixin (Example 5), 4'-Demethyl-4ß-[2"-hydroxypropylamino]-4-desoxypodophyllotoxin (Example 10), and 4'-Demethyl-4ß-[1"-methyl-2"-hydroxyethylamino]-4-desoxypodophyllotoxin (Example 11), 4'-Demethyl-4ß-[2"-aminoethoxy]-4-desoxypodophyllotoxin (Example 13); 4'-Demethyl-4ß-[3"-hydroxyanilinyl]-4-desoxypodophyllotoxin (Example 33), 4'-Demethyl-4ß-[2"-hydroxyanilinyl]-4-desoxypodophyllotoxin (Example 34), 4'-Demethyl-4ß-[4"-hydroxyanilinyl]-4-desoxypodophyllotoxin (Example 35), 4'-Demethyl-4β-[3"-fluoroanilinyl]-4-desoxypodophyllotoxin (Example 26); 4'-Demethyl-4β-[4"-fluoroanilinyl]-4-desoxypodophyllotoxin (Example 28); 4'-Demethyl-4β-[4"-chloroanilinyl]-4-desoxypodophyllotoxin (Example 38); 4'-Demethyl-4β-anilinyl-4-desoxypodophyllotoxin (Example 19); 4'-Demethyl-4β-[4"-cyanoanilinyl]-4-desoxypodophyllotoxin

(Example 20); 4'-Demethyl-4β-[3"-cyanoanilinyl]-4-desoxypodophyllotoxin (Example 21); 4'-Demethyl-4β-[4"-ethoxycarbonylanilinyl]-4-desoxypodophyllotoxin (Example 22); 4'-Demethyl-4β-[4"-morpholinoanilinyl]-4-desoxypodophyllotoxin (Example 23); 4'-Demethyl-4β-[3",4"-methylenedioxyanilinyl]-4-desoxypodophyllotoxin (Example 24); 4'-Demethyl-4β-[3",4"-dimethoxyanilinyl]-4-desoxypodophyllotoxin (Example 25); 4'-Demethyl-4β-[3"-pyridylamino]-4-desoxypodophyllotoxin (Example 30); 4'-Demethyl-4β-[2"-pyridylamino]-4-desoxypodophyllotoxin (Example 31); and 4'-Demethyl-4β-[3"-quinolinylamino]-4-desoxypodophyllotoxin (Example 32) equals or exceeds that of etoposide. In addition, as shown in Tables I and III, the DNA strand breakage abilities of 4'-Demethyl-4ß-[2"-hydroxyethylamino]-4-desoxypodophyllotoxin (Example 5), 4'-Demethyl-4ß-[2"-methoxyethylamino]-4-desoxypodophyllotoxin (Example 8), 4'-Demethyl-4ß-[2"-hydroxypropylamino]-4-desoxy-podophyllotoxin (Example 10), and 4'-Demethyl-4ß-[1"-methyl-2"-hydroxyethylamino]-4-desoxypodophyllotoxin (Example 11), 4'-Demethyl-4ß-[2"-aminoethoxy]-4-desoxypodophyllotoxin (Example 13);4'-Demethyl-4ß-[3-hydroxyanilinyl]-4-dexoypodophyllotoxin (Example 33), 4'-Demethyl-4ß-[2-hydroxyanilinyl]-4-desoxypodophyllotoxin (Example 34), 4'-Demethyl-4ß-[4-hydroxyanilinyl]-4-desoxypodophyllotoxin (Example 35), 4'-Demethyl-4β-[3"-fluoroanilinyl]-4-desoxypodophyllotoxin (Example 26); 4'-Demethyl-4β-[2"-fluoroanilinyl]-4-desoxypodophyllotoxin (Example 27); 4'-Demethyl-4β-[4"-fluoroanilinyl]-4-desoxypodophyllotoxin

(Example 28); 4'-Demethyl-4β-[3",5"-difluoroanilinyl]-4-desoxypodophyllotoxin (Example 29); 4'-Demethyl-4β-anilinyl-4-desoxypodophyllotoxin (Example 19); 4'-Demethyl-4β-[4"-cyanoanilinyl]-4-desoxypodophyllotoxin (Example 20); 4'-Demethyl-4β-[3"-cyanoanilinyl]-4-desoxypodophyllotoxin (Example 21); 4'-Demethyl-4β-[4"-ethoxycarbonylanilinyl]-4-desoxypodophyllotoxin (Example 22); 4'-Demethyl-4β-[4"-morpholinoanilinyl]-4-desoxypodophyllotoxin (Example 23); 4'-Demethyl-4β-[3",4"-methylenedioxyanilinyl]-4-desoxypodophyllotoxin (Example 24); 4'-Demethyl-4β-[3",4"-dimethoxyanilinyl]-4-desoxypodophyllotoxin (Example 25); 4'-Demethyl-4β-[3"-pyridylamino]-4-desoxypodophyllotoxin (Example 30); 4'-Demethyl-4β-[3"-quinolinylamino]-4-desoxypodophyllotoxin (Example 32); and 4'-Demethyl-4β-[4"-bromoanilinyl]-4-desoxypodophyllotoxin (Example 40) greatly exceeds that of etoposide.

Table II compares the relative DNA topoisomerase II inhibitory activity of several compounds of the present invention with etoposide. As shown in Table II, the compounds tested exhibited inhibitory activity exceeding that of etoposide by two to eight times.

Preparation of compounds within the scope of the present invention appear in the following examples.

EXAMPLE 1

Preparation of 4'-Demethylepipodophyllotoxin.

5 g. (12.1 mmol) of podophyllotoxin were dissolved in 75 ml of anhydrous CH_2Cl_2. Dry hydrogen bromide gas was then bubbled through the solution to saturation. The reaction

mixture was then capped and allowed to stand at room temperature for 48 hours. Removal of the solvent yielded a residue which was then treated with 25 ml of water, 50 ml of acetone and 5 g. of $BaCO_3$, and refluxed for one hour. The reaction mixture was extracted with chloroform and chromatographed on a silica gel column. The product was obtained by elution with chloroform-methanol (30:1) and recrystallized from CH_2Cl_2/ethylether to give 2.5 g. (52%) of 4'-Demethylepipodophyllotoxin. Spectral data agreed with that described by Kuhn, M., Keller-Julsen, C., and von Wartburg, A., Helv. Chim. Acta, 52, 944 (1969), which is herein specifically incorporated by reference. (See Scheme 1)

EXAMPLE 2

Preparation of 4'-Demethylpodophyllotoxin.

4'-Demethylpodophyllotoxin was obtained using the silica gel column of Example 1 by further elution with chloroform-methanol (30:1). The product obtained by elution was crystallized from acetone in 5% (0.5 g) yield. Spectral data agreed with that described by Kuhn, M., and von Wartburg, A., Helv. Chim. Acta, 52, 948 (1969) (hereinafter Kuhn and von Wartburg), which is herein specifically incorporated by reference.

EXAMPLE 3

Preparation of 4'-Demethyl-4ß-amino-4-desoxy-podophyllotoxin. (Scheme II)

A. Preparation of 4'-O-Carbobenzoxyepipodo-phyllotoxin.

A solution of 2 g. of 4'-Demethy-lepipodophyllotoxin (5 mmol) in 200 ml of anhydrous dichloromethane was cooled in an ice

bath, and treated with 2.5 ml of triethylamine (18 mmol), and 2.5 ml of carbobenzoxychloride (17.5 mmol). The reaction mixture was stirred at room temperature for 2 hours after which time 100 ml of water was added. The organic layer was dried using $MgSO_4$, concentrated, and purified using silica gel column chromatography. The product was obtained upon elution with chloroform and recrystallized from chloroform/ethanol to give 2.4 g. (89%). Spectral data agreed with that described by Kuhn and von Wartburg.

B. Preparation of 4'-O-Carbobenzoxy-4-epiazidopodophyllotoxin.

A solution of 3 g. (5.6 mmol) of 4'-O-Carbobenzoxy-4-epipodophyllotoxin (the product of Example 3) in 100 ml of anhydrous methylene chloride was cooled in an ice bath and treated successively with 1.5 ml (10.8 mmol) of triethylamine, and 1.2 ml (15.5 mmol) of methanesulfonylchloride. The ice bath was then removed and the reaction mixture was stirred at room temperature for one hour. This mixture was then evaporated in vacuo to dryness, and 40 ml of anhydrous DMF was added along with 3 g. (46 mmol) of sodium azide. The reaction mixture was stirred overnight at room temperature and then partitioned between water (100 ml) and ethylacetate. The organic layer was washed with water, dried using $MgSO_4$, and concentrated to yield a crude residue, which was checked by TLC and NMR analyses to be a mixture of 4α- and 4ß-azido isomers (ca.1:3). Crystallization from chloroform/ethanol provided the pure ß-isomer 4'-O-Carbobenzoxy-4-epiazidopodophyllotoxin (2.3 g, 73%) having the following properties:

mp.202-204° C; MS, m/z 559 (M^+), 424, and 382; IR (KBr) 2950, 2900, 2100 (azide), 1770 (carbonate C=O), 1745 (lactone C=O), 1600, and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.40 (m, 5H, cbz aromatic), 6.82 (s, 1H, 5-H), 6.58 (s, 1H, 8-H), 6.27 (s, 2H, 2'.6'-H), 6.03 (ABq, J=2.4 Hz, 2H, O-CH_2-O), 5.25 (s, 2H, OCH_2Ph), 4.77 (d, J=4 Hz, 1H, 4-H), 4.65 (d, J=5 Hz, 1H, 1-H), 4.31 (d, J=9 Hz, 2H, 11, 11'-H), 3.66 (s, 6H, 3',5'-OCH_3), 3.2(dd, J=5,14 Hz, 1H, 2-H), and 2.90 (m,1H, 3-H); Anal. ($C_{29}H_{25}O_9N_3 \cdot 1/2\ H_2O$), C.H.

C. Preparation of 4'-Demethyl-4β-amino-4-desoxy-podophyllotoxin.

500 mg of 10% palladium on carbon was added to a solution of the crude 4'-demethyl-4-azidopodophyllotoxin (2.3g, 4.1 mmol), obtained according to steps A and B, and 200 ml of ethylacetate. This mixture was shaken under 40 psi of hydrogen for four hours. The reaction mixture was then filtered over celite and the filtrate evaporated in vacuo. The residue was chromatographed on a silica gel column and eluted first with a chloroform/ethylacetate (2:1) solvent system to remove the non-polar products. Further elution with a chloroform/methanol (19:1) mobile phase yielded 0.85g, (52%) of the desired product. The product was then crystallized from methylene chloride/ethylether and had the following properties: mp 132-135° C; MS m/z 399 (M^+); IR (KBr) 3360 (OH), 3290 (primary amine), 2900 (aliphatic C-H), 1745 (lactone), 1590 (aromatic C-H) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.81 (s, 1H,5-H), 6.49 (s, 1H, 8-H), 6.30 (s, 2H, 2',6'-H), 5.96 (ABq, J=1 Hz, 2H, OCH_2O), 5.3 (s, 1H, OH, D_2O exchangeable), 4.55 (d, J=5.2 Hz, 1H, 1-H), 4.28

(d, J=9.5 Hz, 2H, 11,11'-H), 4.17 (d, J=4.1 Hz, 1H, 4-H) 3.77 (s, 6H, 3',5'-OCH_3), 3.28 (dd, J=5.2,14 Hz, 1H, 2-H), and 2.85 (m, 1H, 3-H); Anal. ($C_{21}H_{21}O_7N \cdot H_2O$), C.H.

EXAMPLE 4

Preparation of 4'-Demethyl-4α-amino-4-desoxy-podophyllotoxin.

4'-Demethyl-4α-amino-4-desoxy-podophyllotoxin was obtained from the column used in Example 3 by further elution with a chloroform/methanol (19:1) mobile phase. The pure product (0.34g, 20%) was crystallized from methylene chloride/ethylether and had the following properties: mp 133-135° C; MS m/z 399 (M^+); IR (KBr) 3360 (OH), 3295 (NH_2), 2900 (aliphatic C-H), 1743 (lactone), 1590 (aromatic C-H) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.14 (s, 1H, 5-H), 6.54 (s, 1H, 8-H), 6.20 (s, 2H, 2',6'-H), 6.00 (ABq, J=1 Hz, 2H, OCH_2O), 4.63 (d, J=5.1 Hz, 1H, 1-H), 4.61 (d, J=9.0 Hz, 1H, 11α-H), 4.07 (dd, J=9.0,10.4 Hz, 1H, 11β-H), 3.83 (d, J=10.3 Hz, 1H, 4-H), 3.81 (s, 6H, 3',5'-OCH_3), 2.85 (dd, J=5.1,14.1 Hz, 1H, 2-H), and 2.57(m, 1H, 3-H); Anal. ($C_{21}H_{21}O_7N \cdot H_2O$), C.H.

EXAMPLES 5 - 12

Preparation of 4-alkylamino-4-desoxy-podophyllotoxins. (Scheme III)

The 4-alkylamino-4-desoxy-podophyllotoxins specified in Examples 5-12 were prepared according to the following procedure. A solution of podophyllotoxin (5 g, 12.1 mmol) in 50 ml of anhydrous methylenechloride was kept at room temperature and dry hydrogen bromide gas was bubbled through the solution until saturation was achieved. The flask was then

capped and allowed to stand for 48 hours after which time dry nitrogen was bubbled through the solution to drive off excess HBr. Then 2 g. of anhydrous $BaCO_3$ and 2 ml of the appropriate amine were added. Vigorous evolution of gas was observed. The mixture was allowed to stand for 5 hours at room temperature after which the reaction mixture was filtered, washed with water, dried, and purified via column chromatography. Yields ranged from 5-10%. The products obtained in these examples had the characteristics listed below.

EXAMPLE 5

4'-Demethyl-4β-[2"-hydroxyethylamino]-4-desoxy-podophyllotoxin.

Amorphous powder from CH_2Cl_2-ether : mp 120° C; MS m/z 443 (M^+); IR (KBr) 3420 (NH,OH), 2900 (aliphatic C-H), 1755 (lactone), 1600, and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.82 (s, 1H, 5-H), 6.49 (s, 1H, 8-H), 6.29 (s, 2H, 2',6'-H), 5.97 (ABq, J=1.0,4.4 Hz, 2H, OCH_2O), 4.57 (d, J=5.0 Hz, 1H, 1-H), 4.35 (m, 2H, 11,11'-H), 3.93 (d, J=4.0 Hz, 1H, 4-H), 3.79 (s, 6H, 3',5'-OCH_3), 3.76 (m, 2H, 2"-H), 3.3 (dd, J=5.0,13.5 Hz, 1H, 2-H), 3.09 (m. 1H, 3-H), and 2.75 (m, 2H, 1"-H); Anal. ($C_{23}H_{25}O_6N \cdot 1/2\ H_2O$), C.H.

EXAMPLE 6

4'-Demethyl-4α-[2"-hydroxyethylamino]-4-desoxy-podophyllotoxin.

Crystals from CH_2Cl_2-ether : mp 230-234° C; MS m/z 443 (M^+); IR (KBr) 3425 (NH,OH), 2900 (aliphatic C-H), 1753 (lactone), 1600, and 1475 (aromatic C=C) cm^{-1}; ^1H NMR (CD_3OD) δ 6.83 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.38 (s, 2H, 2',6'-H), 5.92 (ABq, J=1.0,14.3 Hz, 2H, OCH_2O),

4.40 (s, 1H, 11-H), 4.25 (s, 1H, 11'-H), 3.75 (s, 6H, 3',5'- OCH_3), 3.65 (m, 4H, 1 ",2"-H), 3.56 (m, 1H, 1-H), 3.49 (dd, J=6.1,11.3 Hz, 1H, 4-H), 2.87 (ddd, J-5.1, 6.3, 13.5, 1H, 3-H), and 2.67 (dd, J=6.1, 8.2, 1H, 2-H); Anal. ($C_{23}H_{25}O_8N \cdot 1/2\ H_2O$), C.H.

EXAMPLE 7

4'-Demethyl-4ß-propylamino-4-desoxypodophyllotoxin.

Crystals from CH_2Cl_2-ether: mp 153-156°C; MS m/z 441 (M+); IR (KBr) 3470 (OH), 3320 (NH), 1750 (lactone), 1600, and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.77 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.28 (s, 2H, 2',6'-H), 5.95 (ABq, J=1.2,5.0 Hz, 2H, OCH_2O), 4.30 (d, J=5.0 Hz, 1H, 1-H), 4.30 (d, J=4.0 Hz, 1H, 4-H), 4.28(m, 2H, 11,11'-H), 3.78(s, 6H, 3',5'-OCH_3), 3.30 (dd, J=5.0, 13.9 Hz, 1H, 2-H), 2.83 (m, 2H, 1"-H), 2.52 (m, 1H, 3-H), 1.55(m, 2H, 2"-H), and 0.95 (t, J=7.6 Hz, 3H, 3"-H); Anal. ($C_{24}H_{27}O_7N$ $1/2H_2O$), C.H.

EXAMPLE 8

4'-Demethyl-4ß-[2"-methoxyethylamino]-4-desoxy-podophyllotoxin.

Crystals from CH_2Cl_2-ether : mp 202-204° C; MS m/z 457 (M+); IR (KBr) 3440 (OH, NH), 1750 (lactone), 1600, and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.80 (s, 1H, 5-H), 6.44 (s, 1H, 8-H), 6.25 (s, 2H, 2',6'-H), 5.92 (ABq, J=1.0,5.0 Hz, 2H, OCH_2O), 4.50 (d, J=5.4 Hz, 1H, 1-H), 4.28 (m, 2H, 11,11'-H), 3.88 (d, J=4.0 Hz, 1H, 4-H), 3.75 (s, 6H, 3',5'-OCH_3), 3.52 (m, 2H, 2"-H), 3.37 (s, 3H, 3"-H), 3.38 (dd, J=14.4,5.4 Hz, 1H, 2-H), 3.05 (m, 1h, 3-H), and 2.75 (m, 2H, 1"-H); Anal. ($C_{24}H_{27}O_8N \cdot 1/4\ H_2O$), C.H.

EXAMPLE 9

4'-Demethyl-4β-allylamino-4-desoxypodophyllotoxin.

Amorphous powder from CH_2Cl_2-ether : mp 225-228° C; MS m/z 439 (M$^+$); IR (KBr) 3340 (OH, NH), 2885 (aliphatic C-H), 1745 (lactone), 1600, and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.77 (s, 1h, 5-H), 6.49 (s, 1H, 8-H), 6.28 (s, 2H, 2',6'-H), 5.95 (ABq, J=1.0, 4.5 Hz, 2H, OCH_2O), 5.90 (m, 1H, 2"-H), 5.4 (m, 1H, N-H, D_2O exchangeable), 5.22 (dd, J=4.0, 17.5 Hz, 2H, 3"-H), 4.53 (d, J=5.5 Hz, 1H, 1-H), 4.30 (m, 2H, 11,11'-H), 3.88 (d, J=3.6 Hz, 1H, 4-H), 3.75 (s, 6H, 3',5'-OCH_3), 3.30 (dd, J=5.4, 14.4 Hz, 1H, 2-H), 3.30 (m, 1H, 1"-H), and 2.80 (m, 1H, 3-H); Anal. ($C_{24}H_{25}O_7N \cdot 2.2\ H_2O$), C.H.

EXAMPLE 10

4'-Demethyl-4β-[2"-hydroxypropylamino]-4-desoxypodophyllotoxin.

Crystals from CH_2Cl_2-ether : mp 145-150° C; MS m/z 457 (M$^+$); IR (KBr) 3330 (OH, NH), 2890 (aliphatic C-H), 1750 (lactone), 1600, and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.83 (s, 1h, 5-H), 6.47 (s, 1H, 8-H), 6.27 (s, 2H, 2',6'-H), 5.95 (ABq, J=1.0, 6.3 Hz, 2H, OCH_2O), 5.40 (m, 1h, N-H, D_2O exchangeable), 4.54 (d, J=4.6 Hz, 1H, 1-H), 4.30 (m, 2H, 11,11'-H), 3.85 (m, 1H, 2"-H), 3.85 (d, J=3.8 Hz, 1H, 4-H), 3.75 (s, 6H, 3',5'-OCH_3), 3.25 (dd, J=4.6, 13.8 Hz, 1H, 2-H), 2.85 (dd, J=6.8, 12.5 Hz, 1H, 1"-H), 2.82 (m, 1h, 3-H), 2.63 (dd, J=3.8, 12.5 Hz, 1H, 1"-H), and 1.20 (d, J=6.3 Hz, 3H, 3"-H); Anal. ($C_{24}H_{25}O_8N \cdot 1/2\ H_2O$), C.H.

EXAMPLE 11

4'-Demethyl-4β-[1"-methyl-2"-hydroxyethylamino]-4-desoxypodophyllotoxin.

Amorphous powder from CH_2Cl_2-ether : mp 220-225° C; MS m/z 457 (M^+); ^1H NMR ($CDCl_3$) δ 6.89 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.27 (s, 2H, 2',6'-H), 5.95 (ABq, J=1.0, 4.5 Hz, 2H, OCH_2O), 5.40 (m, 1H, N-H, D_2O exchangeable), 4.52 (d, J=4.8 Hz, 1H, 1-H), 4.30 (d, J=9.0 Hz, 2H, 11,11'-H), 4.00 (d, J=4.0 Hz, 1H, 4-H), 3.74 (s, 6H, 3',5'-OCH_3), 3.50 (m, 2H, 2"-H), 3.22 (dd, J=4.8, 13.5 Hz, 1H, 2-H), 2.85 (m, 1H, 3-H), 2.82 (m, 1H, 1"-H)m and 1.05 (d, J=6.3 Hz, 3H, 1'-CH_3); Anal. ($C_{24}H_{29}O_8N \cdot 1/2\ H_2O$), C.H.

EXAMPLE 12

4'-Demethyl-4β-[3"-hydroxypropylamino]-4-desoxy-podophyllotoxin.

Crystals from CH_2Cl_2-ether : mp 193-196° C; MS m/z 457 (M^+); IR (KBr) 3460 (OH) 3320 (NH), 2900 (aliphatic C-H), 1740 (lactone), 1600, and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.75 (s, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.24 (s, 2H, 2',6'-H), 5.94 (ABq, J=1.0,4.4 Hz, 2H, OCH_2O), 4.52 (d, J=5.3 Hz, 1H, 1-H), 4.33 (dd, J=7.9,8.0 Hz, 1H, 11-H), 4.23 (dd, J=8.0,10.7 Hz, 11'-H), 3.78 (d, J=4.0 Hz, 1H, 4-H), 3.73 (s, 6H, 3',5'-OCH_3), 3.72 (t, 2H, 3"-H), 3.21 (dd, J=5.3,14.0 Hz, 1H, 2-H), 3.11 (dd, J=5.9,11.4 Hz, 1H, 1"-H), 2.64 (ddd, J=14.0,7.0,11.0 Hz, 1H, 1"-H) and 1.75 (m, 2H, 2"-H); Anal. ($C_{24}H_{27}O_8N \cdot 3/4\ H_2O$), C.H.

EXAMPLE 13

Preparation of 4'-Demethyl-4β(2"-aminoethoxy)-4-desoxypodophyllotoxin. (Scheme IV)

4'-Demethyl-4ß(2"-aminoethoxy)-4-desoxypodophyllotoxin was prepared according to the following procedure.

A. 4'-Demethyl-4ß-(2"-bromoethoxy)-4-desoxy-podophyllotoxin.

Podophyllotoxin (500 mg) was suspended in anhydrous dichloromethane (15 ml). Dry hydrogen bromide gas was bubbled through the mixture until saturation was achieved. The flask was then capped and allowed to stand at room temperature for 48 h. After bubbling nitrogen gas through the solution to drive off excess hydrogen bromide gas, barium carbonate (1.50 g) and 2-bromoethanol (1 ml) were added and stirred at room temperature for 10 h. The mixture was diluted with dichloromethane, filtered and evaporated to dryness. The syrupy residue was purified by silica gel column chromatography eluting with chloroform-acetone (30 : 1 v/v). For further purification the product was chromatographed on silica gel column using toluene-ethylacetate (5 : 1 v/v) as an eluant. Yield (218 mg). mp 194-195°C. ^{1}H-NMR ($CDCl_3$): δ 6.79 (1H, S, 5-H), 6.56 (1H, S, 8-H), 6.25 (2H, S, 2', 6'-H), 6.00-5.97 (each d, J= 1.2 Hz, OCH_2O), 5.40 (H, S, 4-OH), 4.88 (1H, dd, J= 7.60, 8.34 Hz, 11-H), 4.61 (1H, d, J= 5.2 Hz, 1-H), 4.50 (2H, m), 4.04 (1H, m), 3.80 (1H, m), 3.77 (6H, S, 2 x OCH_3), 3.47 (2H, m), 3.42 (1H, dd, J= 5.39, 14.08 Hz, 2-H), 2.90 (1H, m, 3-H)

Anal. Calcd. for $C_{23}H_{23}BrO_8$: C, 54.45; H, 4.57. Found: C, 54.35; H, 4.60.

B. 4'-Demethyl-4ß-(2"-azidoethoxy)-4-desoxy-podophyllotoxin.

The mixture of 4'-demethyl-4ß-(2-bromoethyl) epipodophyllotoxin (157 mg) and sodium azide (150 mg) in N,N-dimethylformamide (6 ml) was stirred for 10 h at room temperature. Pouring the reaction mixture into water and stirring gave a white precipitate, which was collected by filtration and dried in the air. Recrystallization from chloroform-ether gave pure product (120 mg). mp 215-217°C IR ($CHCl_3$) cm^{-1}: 3538 (OH), 2205 (N_3), 1770 (lactone), 1615 (aromatic C=C) Anal. Calcd. for $C_{23}H_{23}N_3O_8$: C, 58.84; H, 4.93; N, 8.95.
Found: C, 58.78; H, 4.98; N, 9.28.

C. 4'-Demethyl-4ß(2"-aminoethoxy)-4-desoxy-podoplyllotoxin.

A mixture of 4'-Demethyl-4ß-(2"-azidoethyl)epipodophyllotoxin (108 mg) and 10 % palladium in carbon (55 mg) in ethylacetate (20 ml) was stirred under a hydrogen atmosphere for 5 h. After the removal of the catalyst by filtration, the filtrate was evaporated to dryness under reduced pressure. The crude product was purified by silica column chromatography eluting with chloroform-methanol (5 : 1 v/v) to obtain pure material (82 mg) having the following characteristics.
mp 143-145°C
Anal. Cald. for $C_{23}H_{25}NO_8 \cdot H_2O$: C, 58.72; H, 6.00; N, 2.97.
Found: C, 58.74; H, 5.98; N, 2.97.

EXAMPLE 14

Preparation of 4'-Demethyl-4ß-(2"-hydroxy-ethoxy)-4-desoxypodophyllotoxin. (Scheme V)

Podophyllotoxin (200 mg) was suspended in anhydrous dichloromethane (15 ml). Dry

hydrogen bromide gas was bubbled through the mixture until saturation was achieved. The flask was then capped and allowed to stand at room temperature for 48 h. After bubbling nitrogen gas through the solution to drive off excess hydrogen bromide gas, anhydrous barium carbonate (500 mg) and ethyleneglycol (500 mg) were added and stirred at room temperature for 10 h. The mixture was diluted with dichloromethane, filtered, washed with water and the organic layer was dried over anhydrous $MgSO_4$. The removal of solvent gave a syrup, which was purified by silica gel column chromatography eluting with chloroform-acetone (30 : 1 v/v) to obtain pure product (80 mg). The resulting product had the following properties:
^{1}H-NMR ($CDCl_3$): δ 6.82 (1H, S, 5-H), 6.51 (1H, S, 8-H), 6.23 (2H, S, 2', 6'-H), 5.95 (2H, S,OCH_2O), 5.52 (1H, S, OH), 4.70 4.15 (m), 3.72 (s, 6H, 3',5'-OCH_3).

EXAMPLE 15

Preparation of 4'-demethyl-4β-Chloro-4-desoxypodophyllotoxin. (Scheme VI)

Methylsulfide (0.3 ml) and N-chloro-succinimide (60 mg, 0.45 mmole) were added at 0°C to a solution of 4'-demethylpodophyllotoxin (100 mg, 0.25 mmole) in methylene chloride (15 ml). The mixture was stirred for 5 h at 0°C under a nitrogen atmosphere. After the removal of the volatile reagents by evaporation in vacuo, the residue was purified by silica gel chromatography eluting with methylene chloride and acetone to obtain the pure product (82 mg, 78.5%). The product had the following properties:
IR($CHCl_3$) cm^{-1}:3540(OH), 1770(lactone)

^{1}H-NMR($CDCl_3$) δ:6.57 (s, 1H, 8-H), 6.53 (s, 1H, 5-H), 6.30 (s, 2H, 2'6'-H), 6.02, 5.98 (each d, 2H, J=1.3 Hz, OCH_2O), 5.42 (s, 1H, OH), 4.62 (d, 1H, J=5.2 Hz, 1-H), 4.53 (m, 2H, 11-CH_2), 4.45 (d, 1H, J-3.3 Hz, 4-H), 3.80 (s, 6H, 3', 5'-OCH_3), 3.41 (dd, 1H, J-5.3, 14 Hz, 2-H), 2.86 (m, 1H, 3-H)

EXAMPLES 16, 17, AND 18

Preparation of 4, 5, 8, 2'-tetrabromo-4 ß-desoxypodophyllotoxin, 5, 2', 6'-tribromo-4ß-desoxypodophyllotoxin, and 5, 8, 2',6'-tetrabromo-4ß-desoxypodophyllotoxin. (Scheme VII)

The compounds of Examples 16,17, and 18 were prepared according to the following procedure. To a solution of podophyllotoxin (200 mg, 0.54 mMol) in $CHCl_3$ (7 ml) was added bromine (0.5 ml, 9.70 mMol). After stirring for 2 h. at room temperature, the mixture was poured into ice-water, and extracted with $CHCl_3$, followed by washing with 5% sodium hydrosulfite to remove excess bromine. The organic layer was dried over anhydrous magnesium sufate, filtered, and concentrated in vacuo to yield a crude product. This was purified by use of preparative TLC ($CHCl_3$-acetone 15:1) to obtain the compounds of Example 16, 17, and 18.

EXAMPLE 16

4, 5, 8, 2'-tetrabromo-4ß-desoxypodophyllotoxin: 16 mg; NMR ($CDCl_3$) δ 6.21 (d, J=1.0 Hz, 2H, OCH_2O), 5.85 (s, 1H, 6'-H), 5.74 (d, J=3.2 Hz, 1H, 4-H), 5.45 (d, J=6.4 Hz, 1H, 1-H), 4.49 (m, 2H, 11,11'-H), 3.95 (s, 3H, 3'-OCH_3), 3.92 (s, 3H, 4'-OCH_3), 3.68 (s, 3H, 5'-OCH_3), 3.44 (dd, J=6.4, 14.4 Hz, 1H, 2-H) and

3.35 (m, 1H, 3-H); IR ($CHCl_3$) no OH band, 1785 (lactone) cm^{-1}.

EXAMPLE 17

5, 2', 6'-tribromo-4β-desoxypodophyllotoxin: 60 mg: NMR ($CDCl_3$) δ 6.33 (s, 1H, 8-H), 6.04 (d, J=1.0Hz, 2H, OCH_2O), 5.72 (d, J=9.5 Hz, 1H, 1-H), 5.25 (br.s, 1H, 4-H), 4.40 (m, 2H, 11, 11'-H), 4.95 (s, 3H, 3'-OCH_3), 3.94 (s, 3H, 5'-OCH_3), 3.81 (s, 3H, 4'-OCH_3), 3.70 (m, 1H, 3-H), and 3.55 (dd, J=9.5, 15.0 Hz, 1H, 2-H: IR ($CHCl_3$) 3600 (OH), 1776 (lactone) CM^{-1}.

EXAMPLE 18

5, 8, 2', 6'-tetrabromo-4β-desoxydophyllotoxin: 16 mg; NMR ($CDCl_3$) δ 6.14 (d, J=2.2, 2H, OCH_2O), 5.70 (d, J=7.6, 1H, 1-H), 5.21 (d, J=2.8 Hz, 1H, 4-H), 4.40 (m, 2H, 11,11'-H 3.93 (s, 6H, 3'and 5'-OCH_3), 3.78 (s, 3H, 4'-OCH_3), 3.65 (m, 1H, 3-H) and 3.49 (dd, J=9.5, 15 Hz, 1H, 2-H); IR ($CHCl_3$) 3600 (OH), 1776 (lactone) cm^{-1}.

16, $R_1=R_2=R_3=R_5=Br$, $R_4=H$
17, $R_1=OH$, $R_2=R_4=R_5=Br$, $R_3=H$
18, $R_1=OH$, $R_2=R_3=R_4=R_5=Br$

EXAMPLE 19 - 41

Preparation of 4'demethyl-4β-(arylamino)-4-desoxypodophyllotoxins (19-41). (Scheme VIII)

The 4'-demethyl-4β-(arylamino)-4-deoxypodopohyllotoxins specified in examples 19 - 41 were prepared according to the following procedure:

A solution of 4'-demethyl-epipodophyllotoxin (10 g, 24 mmol) in 250 ml of dry dichloromethane was kept at 0°C, and dry hydrogen bromide gas was bubbled through the solution. After 30 min., nitrogen was bubbled through the solution to drive off excess hydrogen bromide. The solution was then evaporated in vacuum to dryness by means of azeotropic distillation with benzene.

The desired product (11.5 g) was obtained and then used for the next step reaction without any further purification.

Spectral data agreed with that described by M. Kuhn and A. Von Wartburg. Helv. Chim. Acta, 52, 944 (1969).

A solution containing 4'-demethyl-4β-bromo-4-deoxypodophyllotoxin (300 mg, 0.65 mmol), barium carbonate (153 mg, 0.78 mmol) and the appropriate arylamines (0.78 mmol) in 7 ml of dry 1, 2-dichloroethane was stirred overnight at room temperature. The reaction mixture was filtered, diluted with ethyl acetate, washed with water, dried and purified via column chromatography. The products (19-41) obtained in the examples had the characteristics listed below.

EXAMPLE 19

4'-Demethyl-4β-anilinyl-4-desoxypodophyllotoxin.

Crystals from methanol; mp 172-173°, $[\alpha]^{25}_{D}$-120° (C=1.0, $CHCl_3$); IR (KBr) 3500 (OH), 3360 (NH), 2900 (aliphatic C-H), 1755 (lactone), 1595, 1500 and 1475 (aromatic c=c) cm^{-1}: ^1H NMR $CDCl_3$ δ 7.22 (t, J=7.5 Hz, 2H, 3", 5"-H), 6.80 (m, 2H, 4" -H and 5-H), 6.50 (m, 3H, 2"-H, 6"-H and 8-H), 6.33 (s, 2H, 2', 6'-H), 5.97 (AB_q, J=1.3, 3.6 Hz, OCH_2O), 5.42 (s, 1H, exchangeable,

4'-OH), 4.68 (br, 1H, 4-H), 4.60 (d, J=4.9 Hz, 1-H), 4.38 (t, J=8.4 Hz, 1H, 11-H), 4.01 (t, J=8.4 Hz, 1H, 11-H), 3.85 (br, 1H, exchangeable, NH), 3.79 (s, 6H, 3', 5'-OCH_3), 3.16 (dd, J=5.0, 14.0 Hz, 1H, 2-H), 3.00 (m, 1H, 3-H).

Anal. ($C_{27}H_{25}NO_7$), C.H.N.

EXAMPLE 20

4'-Demethyl-4β-[4"-cyanoanilinyl]-4-desoxypodophyllotoxin.

Crystals from ethanol; mp 187-189°, $[\alpha]^{25}_D$-145° (C=1.0, $CHCl_3$); IR (KBr) 3500 (OH), 3360 (NH), 2890 (aliphatic C-H), 2210 (lactone), 1600, 1510 and 1475 (aromatic C=C) cm^{-1}: ^1H NMR $CDCl_3$ δ 7.50 (d, J=8.7 Hz, 2H, 3", 5"-H), 6.74 (s, 1H, 5-H), 6.57 (d, J=8.7 Hz, 2H, 2", 6"-H), 6.55 (s, 1H, 8-H), 6.32 (s, 2H, 2', 6'-H), 5.99 (AB_q, J=1.2, 8.3 Hz, 2H, OCH_2O), 5.44 (s, 1H, exchangeable, 4'-OH), 4.78 (m, 1H, exchangeable, NH), 4.63 (d, J=4.2 Hz, 1H, 4-H), 4.36 (m, 2H, 11-H), 3.85 (m, 1H, 1-H), 3.79 (s, 6H, 3', 5'-OCH_3), 3.09 (dd, 1H, 2-H), 3.05 (m, 1H, 3-H).

Anal. ($C_{28}H_{24}NO_7$), C.H.N.

EXAMPLE 21

4'-Demethyl-4β-[3"-cyanoanilinyl]-4-desoxypodophyllotoxin.

Crystals from methanol; mp 191-192°, $[\alpha]^{25}_D$-117° (C=0.33, $CHCl_3$); IR (KBr) 3450 (OH), 3360 (NH), 2900 (aliphatic C-H), 2225 (CN), 1750 (lactone), 1595, 1500 and 1450 (aromatic C=C) cm^{-1}: ^1H NMR $CDCl_3$ δ 7.31 (t, J=7.6 Hz, 5"-H), 7.07 (d, J=7.6 Hz, 4"-H), 6.80 (d, 2H, 2"-H and 6"-H), 6.74 (s, 1H, 5-H), 6.55 (s, 1H, 8-H), 6.33 (s, 2H, 2', 6'-H), 6.00 (d, J=7.0 Hz, 2H, OCH_2O), 5.48 (s, 1H exchangeable, 4'-OH), 4.69 (d, J=3.8 Hz, 1H, 4-H), 4.62 (d, J=4.5 Hz, 1H, 2-H), 4.41

(t, J=8.5 Hz, 1H, 11-H), 3.92 (t, J=8.5 Hz, 1H, 11-H), 3.81 (s, 6H, 3', 5'-OCH_3), 3.14-3.00 (m, 2H, 2-H and 3-H).

Anal. ($C_{21}H_{24}N_2O_7$)•1/2 H_2O, C.H.N.

EXAMPLE 22

4'-Demethyl-4β-[4"-ethoxycarbonylanilinyl]-4-desoxypodophyllotoxin.

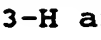
Crystals from ethanol; mp 270-271°, $[\alpha]^{25}_D$-145° (C=0.33, $CHCl_3$); IR (KBr) 3500 (OH), 3370 (NH), 2940 (aliphatic C-H), 1762 (lactone), 1695 (ester), 1610, 1520 and 1480 (aromatic C=C) cm^{-1}: ^1H NMR $CDCl_3$ δ 7.92 (d, J=8.8 Hz, 2H, 3", 5"-H), 6.77 (s, 1H, 5-H), 6.55 (d, J=8.8 Hz, 2H, 2", 6"-H), 6.54 (s, 1H, 8-H), 6.33 (s, 2H, 2', 6'-H), 5.99 (AB_q, J=1.1, 8.2 Hz, 2H, OCH_2O), 5.44 (s, 1H, exchangeable, 4'-OH), 4.78 (d, J=3.3 Hz, 1H, 4-H), 4.62 (d, J=4.5 Hz, 1H, 1-H), 4.40 (m, 2H, 4-H and 11-H), 4.37 (q, J=7.1 Hz, 2H, $CO_2\underline{CH_2}CH_3$), 4.32 (d, J=7.1, 1H, exchangeable, NH), 3.92 (t, J=7.5 Hz, 1H, 11-H), 3.80 (s, 6H, 3', 5'-OCH_3), 3.10 (dd, 1H, 2-H), 3.08 (m, 1H, 3-H), 1.38 (t, J=7.1 Hz, 3H, $CO_2CH_2\underline{CH_3}$).

Anal. ($C_{30}H_{29}NO_9$), C.H.N.

EXAMPLE 23

4'-Demethyl-4β-[4"-morpholinoanilinyl]-4-desoxypodophyllotoxin.

Crystals from ethanol; mp 235-237°, $[\alpha]^{25}_D$-129° (C=1, $CHCl_3$); IR (KBr) 3500 (OH), 3300 (NH), 2880 (aliphatic C-H), 1755 (lactone), 1620, 1510 and 1475 (aromatic C=C) cm^{-1}: ^1H NMR $CDCl_3$ δ 6.86 (d, J=9.5 Hz, 2H, 3", 5"-H), 6.76 (s, 1H, 5-H), 6.52 (br, 3H, 8-H and 2", 6"-H), 6.35 (s, 2H, 2', 6'-H), 5.96 (d, J=66.7 Hz, 2H, OCH_2O), 5.44 (s, 1H, exchangeable, 4'-OH), 4.61 (m, 2H, 4-H and 1-H), 4.37 (t, J=7.0 Hz, 1H, 11-H), 4.08 (t, J=7.0 Hz, 1H, 11-H), 3.82 (br, 4H,

), 3.80 (s, 6H, 3', 5'-OCH_3), 3.22-2.90 (m, 2H, 2-H, 3-H and 4H, );

Anal. ($C_{31}H_{32}N_2O_8$), C.H.N.

EXAMPLE 24

4'-Demethyl-4β-[3", 4"-(methylenedioxy)anilinyl]-4-desoxypodophyllotoxin.

Crystals from methanol; mp 247-249°, $[\alpha]^{25}_D$-126° (C=1, $CHCl_3$); IR (KBr) 3500 (OH), 3340 (NH), 2900 (aliphatic C-H), 1752 (lactone), 1605, 1496 and 1475 (aromatic C=C) cm^{-1}: ^1H NMR $CDCl_3$, δ 6.76 (s, 1H, 5-H), 6.68 (d, J=8.1 Hz, 1H, 5"-H), 6.52 (s, 1H, 8-H), 6.33 (s, 2H, 2', 6'-H), 6.17 (d, J=1.2 Hz, 1H, 2"-H), 5.96 (q, J=1.2, 8.1 Hz, 3H, 6"H and OCH_2O), 5.90 (s, 2H, 7"-H), 5.43 (s, 1H exchangeable, 4'-OH), 4.59 (d, J=4.9 Hz, 1H, 4-H), 4.56 (d, J=3.9 Hz, 1H, 1-H), 4.37 (t, 1H, 11-H), 4.05 (t, 1H, 11-H), 3.79 (s, 6H, 3', 5'-OCH_3), 3.15 (dd, 1H, 2-H), 2.95 (m, 1H, 3-H).

Anal. ($C_{29}H_{25}NO_8$), C.H.N.

EXAMPLE 25

4'-Demethyl-4β-[3", 4"-dimethoxyanilinyl]-4-desoxypodophyllotoxin.

Crystals from methanol; mp 233-234° (dec); $[\alpha]^{25}_D$-118° (C=1, $CHCl_3$); IR (KBr) 3500 (OH), 3360 (NH), 2920 (aliphatic C-H), 1770 (lactone), 1605, and 1505 (aromatic C=C) cm^{-1}: ^1H NMR $CDCl_3$, δ 6.78 (s, 1H, 5-H), 6.75 (d, J=8.5 Hz, 1H, 5"-H), 6.53 (s, 1H, 8-H), 6.34 (s, 2H, 2', 6'-H), 6.17 (s, 1H, 2"-H), 6.05 (d, J=8.5 Hz, 1H, 6"-H), 5.96 (d, J=2.4 Hz, 2H, OCH_2O), 5.43 (s, exchangeable, 4'-OH), 4.60 (d, 2H, 4-H and 1-H), 4.38 (t, J=8.3 Hz, 1H, 11-H), 4.05

(t, J=8.3 Hz, 1, 11-H), 3.83 (s, 3H, 4"-OCH_3), 3.81 (s, 3H, 3"-OCH_3), 3.80 (s, 6H, 3', 5'-OCH_3), 3.18 (dd, J=5.0, 14.0 Hz, 1H, 2-H), 2.96 (m, 1H, 3-H).

Anal. ($C_{28}H_{25}NO_9$), C.H.N.

EXAMPLE 26

4'-Demethyl-4β-[3"-fluoroanilinyl]-4-desoxypodophyllotoxin.

Crystals from methanol; mp 201-203°C (dec.);$[\alpha]^{25}D$-132° (c = 1, $CHCl_3$); IR (KBr) 3500 (OH), 3360 (NH), 2900 (aliphatic C-H), 1750 (lactone), 1605, 1500 1475 (aromatic C=C) cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 7.15 (t, J = 7.4 Hz, 1H, 5"-H), 6.76 (s, 1H, 5-H), 6.53 (s, 1H, 8-H), 6.49 (dd, H = 1.2, 7.4 Hz, 1H, 4"-H), 6.40 (s, 2H, 2', 6'-H), 6.32 (d, J = 1.2 Hz, 1H, 2"-H), 6.24 (dd, J = 1.2, 7.4 Hz, 1H, 6"-H), 5.97 (ABq, J = 1.2, 7.9 Hz, 2H OCH_2O), 5.44 (s, 1H, exchangeable, 4'-OH), 4.67 (s, 1H, exchangeable, NH), 4.63 (d, J = 4.0 Hz, 1H, 4-H), 4.59 (d, J = 5.0 Hz, 1H, 1-H), 4.39 (t, J = 8.5 Hz, 1H, 11-H) 3.98 (t, J = 8.5 Hz, 1H, 11-H), 3.79 (s, 6H, 3',5'-OCH_3), 3.11 (dd, J = 5.0, 14.0 Hz, 1H, 2-H), 3.00 (m, 1H, 3-H). Anal. calcd for $C_{27}H_{24}FNO_7$:C,65.71;H, 4.90; N, 2.84. Found: C, 66.81; H, 4.94; N, 2.79.

EXAMPLE 27

4'-Demethyl-4β-[2"-fluoroanilinyl]-4-desoxypodophyllotoxin.

Crystals from methanol; mp 197-198°C;$[\alpha]^{25}D$-128° (c = 0.25, $CHCl_3$); IR (KBr) 3500 (OH), 4480 (NH), 2890 (aliphatic C-H), 1755 (lactone), 1610, 1505 and (aromatic C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.04 (m, 2H, 3",6"-H), 6.76 (s, 1H, 5H), 6.72 (m, 1H 5"-H), 6.60 (t, J = 7.2 Hz, 1H, 4"-H), 6.54 (s, 1H, 8-H), 6.34 (s, 2H,

2',6'-H), 5.97 (d, J = 7.3 Hz, 2H, OCH_2O), 5.46 (s, 1H, exchangeable, 4'-OH), 4.69 (d, J = 4.2 Hz, 1H, 4-H), 4.62 (d, J = 4.9 Hz, 1H, 1-H), 4.38 (t, J = 8.2 Hz, 1H, 11-H) 4.10 (t, 1H, exchangeable, NH), 3.82 (t, J = 8.2 Hz, 1H, 11-H) 3.79 (s, 6H, 3',5'-OCH_3), 3.15 (dd, J = 5.0, 14.0 Hz, 1H, 2-H), 3.00 (m, 1H, 3-H). Anal. calcd for $C_{27}H_{24}FNO_7$: C, 65.71; H, 4.90; N, 2.84. Found: C, 66.80; H, 4.95; N, 2.84.

EXAMPLE 28

4'-Demethyl-4β-[4"-fluoroanilinyl]-4-desoxypodophyllotoxin.

Crystals from ethanol; mp 176-177°C; $[\alpha]^{25}D$-100° (c = 0.8, $CHCl_3$); IR (KBr) 3540 (OH), 3420 (NH), 2900 (aliphatic C-H), 1740 (lactone), 1610, 1500 1480 (aromatic C=C) cm^{-1}; ^1HNMR ($CDCl_3$) δ 6.94 (t, J = 6.7, 2-H, 3",5",-H), 6.75 (s, 1H, 5-H), 6.53 (s, 1H, 8H), 6.49 (q, J = 2.2, 6.2 Hz, 2H, 2",6"-H), 6.33 (s, 2H, 2',6'-H), 5.96 (ABq, J = 1.2, 7.5 Hz, 2H OCH_2O), 5.43 (s, 1H, exchangeable, 4'-OH), 4.60 (d, 2H, 4-H and 1-H), 4.37 (t, J = 7.5 Hz, 1H, 11-H) 3.99 (t, J = 7.5 Hz, 1H, 11-H), 3.79 (s, 6H, 3',5'-OCH_3), 3.73 (br, 1H, exchangeable, NH), 3.13 (t, J = 5.0, 14.0 Hz, 1H, 2-H), 3.00 (m, 1H, 3-H). Anal. $C_{27}H_{24}FNO_7$), C.H.N.

EXAMPLE 29

4'-Demethyl-4β-[3", 5"-difluoroanilinyl]-4-desoxypodophyllotoxin.

Crystals from ethanol; mp 180-183°C; $[\alpha]^{25}D$-132° (c = 0.33, $CHCl_3$); IR (KBr) 3500 (OH), 3370 (NH), 2890 (aliphatic C-H), 1750 (lactone), 1620, 1590, 1500 and 1475 (aromatic C=C) cm^{-1}; ^1HNMR ($CDCl_3$) δ 6.75 (s, 1H, 5-H), 6.54 (s, 1H, 8-H), 6.32 (s, 2H, 2',6'-H), 6.23 (m,

1H, 4"-H), 6.07 (m, 2H, 2",6"-H), 5.98 (ABq, J = 1.3, 9.0 Hz, 2H OCH_2O), 5.45 (s, 1H, exchangeable, 4'-OH), 4.61 (m, 2H, 4-H and 1-H), 4.39 (t, J = 8.5 Hz, 1H, 11-H) 4.10 (d, J = 6.1 Hz, 1H, 1H, exchangeable, NH), 3.85 (t, J = 8.5 Hz, 1H, 11-H), 3.81 (s, 6H, 3',5'-OCH_3), 3.08 (dd, J = 4.8, 14.1 Hz, 1H, 2-H), 3.02 (m, 1H, 3-H). Anal. $C_{27}H_{23}NF_2O_9$). C.H.N.

EXAMPLE 30

4'-Demethyl-4β-[3"-pyridylamino]-4-desoxypodophyllotoxin.

Crystals from ethanol; mp 179-181° (dec); $[\alpha]^{25}_D$-99° (C=0.33, $CHCl_3$); IR (KBr) 3500 (OH), 3350 (NH), 2900 (aliphatic C-H), 1765 (lactone), 1575, 1500 and 1470 (aromatic C=C=N) cm^{-1}: ^1H NMR $CDCl_3$ δ 8.08 (d, J=5.5 Hz, 1H, 6"-H), 8.02 (br, 1H, 2"-H), 7.16 (m, 1H, 5"-H), 6.85 (dd, 1H, 4"-H), 6.75 (s, 1H, 5-H), 6.55 (s, 1H, 8-H), 6.32 (s, 2H, 2', 6'H), 5.98 (AB_q, J=1.3, 7.3 Hz, 2H, OCH_2O), 4.65 (d, J=4.9 Hz, 1H, 4-H), 4.60 (m, 1H, 1-H), 4.20 (t, J=8.2 Hz, 1H, 11-H), 3.90 (m, 2H, 11-H and NH), 3.80 (s, 6H, 3', 5'-OCH_3), 3.18 (dd, J=5.0, 14.1 Hz, 1H, 2-H), 3.03 (m, 1H, 3-H).

Anal. ($C_{26}H_{24}N_2O_7$) 1/2 H_2O, C.H.N.

EXAMPLE 31

4'-Demethyl-4β-[2"-pyridylamino]-4-desoxypodophyllotoxin.

Crystals from ethanol; mp 215-218° (dec); $[\alpha]^{25}_D$-82° (C=0.33, $CHCl_3$); IR (KBr) 3500 (OH), 3360 (NH), 2950 (aliphatic C-H), 1760 (lactone), 1690, 1645, 1600 and 1460 (aromatic C=C=N) cm^{-1}: ^1H NMR $CDCl_3$ δ 8.11 (d, 1H, 4"-H), 7.45 (m, 1H, 4"-H), 6.81 (s, 1H, 5-H), 6.67 (m, 1H, 5"-H), 6.55 (s, 1H, 8-H), 6.45 (d, 1H, 3"-

H), 6.34 (s, 2H, 2', 6'-H), 5.97 (AB_q, J=1.3, 6.7 Hz, 2H, OCH_2O), 5.43 (br, 1H, exchangeable, 4'-OH), 5.35 (m, 1H, exchangeable, NH), 4.60 (d, J=4.2 Hz, 1H, 4-H), 4.24 (m, 2H, 1-H and 11NH), 3.85 (m, 1H, 11H), 3.78 (s, 6H, 3', 5'-OCH_3), 3.05 (m, 2H, 2-H and 3-H).

Anal. ($C_{26}H_{24}N_2O_7$), 1/2 H_2O, C.H.N.

EXAMPLE 32

4'-Demethyl-4β-[3"-quinolinylamino]-4-desoxypodophyllotoxin.

Crystals from ethanol-ether; mp 243-246° (dec); $[\alpha]^{25}_D$-179° (C=0.5, $CHCl_3$); IR (KBr) 3460 (OH), 3380 (NH), 2900 (aliphatic C-H), 1775 (lactone), 1605, 1510 and 1480 (aromatic C=C=N) cm^{-1}: ^1H NMR $CDCl_3$ δ 8.46 (d, J=2.9 Hz, 2"-H), 7.97 (m, 1H, 4"-H), 7.65 (m, 1H, 7"-H), 7.48 (m, 2H, 5", 6"-H), 6.99 (d, J=2.9 Hz, 8'-H), 6.76 (s, 1H, 5-H), 6.57 (s, 1H, 8-H), 6.35 (s, 2H, 2', 6'-H), 5.99 (AB_q, J=1.1, 8.0 Hz, 2H, OCH_2O), 5.48 (s, 1H, exchangeable, 4'-OH), 4.78 (d, J=4.8 Hz, 1H, 1-H), 4.45 (t, 1H, 11-H), 4.23 (d, 1H, exchangeable, NH), 3.99 (t, 1H, 11-H), 3.81 (s, 6H, 3', 5'-OCH_3), 3.15 (m, 2H, 2-H and 3-H).

Anal. ($C_{30}H_{26}N_2O_7$) 1/2H_2O, C.H.N.

EXAMPLE 33

4'-Demethyl-4ß-[3''-hydroxyanilinyl]-4-desoxypodophyllotoxin.

Amorphous powder from ether: mp 163-166°C; IR (KBr) 3480 (OH), 3380 (NH), 2900 (aliphatic CH), 1750 (lactone), 1590, 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.05(t,J=8Hz,1H,5"-H), 6.78 (s,1H,5-H), 6.52(s,1H,8-H), 6.33(s,2H,2',6'-H), 6.24 (dd,J=2.2, 8Hz,1H,4"-H), 6.15(dd,J=1.7,

8Hz,1H,6"-H), 6.07(t,J=2.2Hz,1H,2"-H), 5.97(d,J=4.4Hz,2H, OCH_2O), 5.43(s, exchangeable), 4.82(s, exchangeable), 4.65 (d,J=3.9Hz,1H,4-H), 4.58(d,J=4.8Hz,1H,1-H), 4.37(t,J=8.7Hz,1H,11-H), 4.0(t,J=8.7Hz,1H,11-H), 3.79(s,6H,3'5'-OCH_3), 3.1(dd, J=4.8, 14.1 Hz, 1H, 2-H), 2.98(m,1H,3-H); MS, m/z=491 (m+). Anal. Calcd for $C_{27}H_{25}NO_8.H_2O$: C,63.65;H,5.30. Found: C,63.35; H,5.44.

EXAMPLE 34

4'-Demethyl-4β-[2''-hydroxyanilinyl]-4-desoxypodophyllotoxin.

Amorphous crystals from ether: mp 175°C; IR (KBr) 3360 (OH, NH), 2900 (aliphatic C-H), 1750 (lactone, 1600, 1475 (aromatic C=C) cm^{-1};^1H NMR ($CDCl_3$) δ 6.88(t,J=7.4H_z,1H,4"-H), 6.78(s,1H,5-H), 6.65(m,2H,3",6"-H), 6.5(m,2H,8-H,5"-H), 6.35(s,2H,2'6'-H), 5.96(AB_q J=1.2Hz,3.5Hz,2H,OCH_2O), 5.44(s.exchangeable), 5.10(s, exchangeable), 4.67(d,J=4Hz,1H,4-H), 4.61(d,J=4.8Hz,1H,1-H), 4.38(t,J=8.5Hz,1H,11-H), 3.98(t,J=8.5Hz,1H,11-H), 3.79(s,6H,3',5'-OCH_3), 3.24 (dd,J=4.8,14Hz,1H,2-H), 3.02(m,1H,3-H), MS, m/z=491(m+). Anal. Calcd for $C_{27}H_{25}NO_8$: C,65.99; H,5.09. Found: C, 65.85; H,5.18.

EXAMPLE 35

4'-Demethyl-4β-[4''-hydroxyanilinyl]-4-desoxypodophyllotoxin.

Amorphous powder from ether mp 162-165°C;IR (KBr) 3525 (OH), 3345 (NH), 3010 (aromatic CH), 2900 (aliphatic CH), 1745 (lactone), 1600, 1475 (aromatic C=C) cm^{-1};^1H NMR (DMSO d_6, D_2O exchange) δ 6.69(s,1H,5-H), 6.55(s,4H,2",3",5",6"-H), 6.48(s,1H,8-H), 6.23(s,2H,2'6'-H),

5.94(d,J=9.7Hz,2H,O-CH2-O), 4.68(d,J=4.3Hz,1H,4-H), 4.46(d,J=5.4Hz,1H,1-H), 4.29(t,J=7.6,1H,11-H), 3.76(t,J=7.6Hz,1H,11-H), 3.61(s,6H,3',5'-OCH_3), 3.28(dd,J=5.4,15.8Hz,1H,2-H), 2.95(m,1H,3-H).

EXAMPLE 36

4'-Demethyl-4ß-[2"-chloroanilinyl]-4-desoxypodophyllotoxin.

Crystals from ethyl acetate/ether, mp 253-255°C;$[\alpha]^{25}$D-90° (c = 1.0, $CHCl_3$); IR (KBr) 3500 (OH), 3450 (NH), 2895 (aliphatic CH), 1751 (lactone), 1590, 1500 1472 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) 7.31 (dd, J = 1.4, 7.9 Hz, 1H, 3"-H), 7.18 (t, J = 8.8, Hz, 1H, 5"-H), 6.76 (s, 1H, 5-H), 6.73 (t, J = 9.0 Hz, 4"-H) 6.58 (d, J = 8.2 Hz, 1H, 6"-H), 6.54 (s, 1H, 8-H), 6.35 (s, 2H, 2',6'-H), 5.98 (ABq, J = 1.2, 4.2 Hz, 2H $OCOCH_2O$), 5.44 (s, 1H, exchangeable, 4'-OH), 4.73 (t, J = 4.9 Hz, 1H, 4-H) 4.64 (d, J = 4.9 Hz, 1H, 1-H), 4.49 (d, J = 6.0 Hz, 1H, exchangeable, NH), 4.36 (t, J = 8.3 Hz, 1H, 11-H), 3.91 (t, J = 8.3, Hz, 1H, 11-H), 3.80 (s, 6H, 3,'5'-OCH_3), 3.17 (dd, J = 4.8, 14.0 Hz, 1H, 2-H), 3.04 (m, 1H, 3-H). Anal. $C_{27}H_{24}ClNO_7$ C.H.N.

EXAMPLE 37

4'-Demethyl-4ß-[3"-chloroanilinyl]-4-desoxypodophyllotoxin.

Crystals from ethyl acetate/ether, mp 174-176°C;$[\alpha]^{25}$D-112° (C = 1.0, $CHCl_3$); IR (KBr) 3500 (OH), 3360 (NH), 2920 (aliphatic CH), 1752 (lactone), 1580, and 1452 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) 7.12 (t, J = 8.1, Hz, 1H, 5"-H), 6.76 (s, 1H, 5-H), 6.74 (dd, J = 1.0, 8.1 Hz, 1H 4"-H), 6.53 (br, 2H, 8-H and 2"-H), 6.42 (dd, J = 1.6, 6.5 Hz, 1H, 6"-H), 6.33 (s, 2H, 2',6'-H),

5.97 (ABq, J = 1.0, 8.7 Hz, 2H OCH_2O), 5.43 (s, 1H, exchangeable, 4'-OH), 4.66 (br, 1H, 4-H), 4.59 (d, J = 4.8 Hz, 1H, 1-H), 4.39 (t, J = 7.7 Hz, 1H, 11-H), 3.99 (t, J = 7.7 Hz, 1H, 11-H) 3.96 (br, 1H, exchangeable, NH), 3.79 (s, 6H, 3,'5'-OCH_3), 3.11 (dd, J = 5.8, 14.0 Hz, H, 2-H), 3.01 (m, 1H, 3-H). Anal. $C_{27}H_{24}ClNO_7$) C.H.N.

EXAMPLE 38

4'-Demethyl-4ß-[4"-chloroanilinyl]-4-desoxypodophyllotoxin.

Crystals from ethyl acetate/ether, mp 253-255°C;$[\alpha]^{25}D$-125° (c = 0.75 $CHCl_3$); IR (KBr) 3500 (OH), 3360 (NH), 2920 (aliphatic CH), 1758 (lactone), 1605, 1590 and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.17 (d, J = 8.7, Hz, 2H, 3",5"-H), 6.74 (s, 1H, 5-H), 6.53 (s, 1H, 8-H), 6.48 (d, J = 8.7 Hz, 2H, 2",6"-H), 6.32 (s, 2'6'-H), 5.96 (ABq, J = 1.0, 6.8 Hz, 2H OCH_2O), 5.43 (s, 1H, exchangeable, 4'-OH), 4.63 (d, J = 4.2 Hz, 1H, 4-H), 4.59 (d, J = 4.9 Hz, 1H, 1-H), 4.38 (t, J = 8.0 Hz, 1H, 11-H) 3.96 (t,J = 8.0 Hz, 1H, 11-H), 3.79 (s, 6H 3,'5'-OCH_3), 3.12 (dd, J = 4.9, 14.1 Hz, H, 2-H), 2.99 (m, 1H, 3-H). Anal. $C_{27}H_{24}ClNO_7$) C.H.N.

EXAMPLE 39

4'-Demethyl-4ß-[3"-bromoanilinyl]-4-desoxypodophyllotoxin.

Crystals from methanol/ether; mp 177-179°C;$[\alpha]^{25}D$-105° (c = 1, $CHCl_3$); IR (KBr) 3450 (OH), 3340 (NH), 2900 (aliphatic CH), 1740 (lactone), 1590, 1500 and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.07 (t, J = 8.0, Hz, 1H, 5"-H), 6.90 (dd, J = 0.9, 7.9 Hz, 1H, 4"-H), 6.75 (s, 1H, 5-H), 6.70 (br, 1H, 2"-H), 6.53 (s, 1H, 8-H), 6.47 (dd, J = 1.7, 8.3 Hz, 1H 6"-H), 6.33

(s, 2H, 2',6'-H), 5.97 (dd, J = 1.2, 9.3 Hz, 2H, OCH_2O), 5.43 (s, 1H, exchangeable, 4'-OH), 4.65 (d, J = 4.2 Hz, 1H, 4-H), 4.60 (d, J = 4.8 Hz, 1H, 1-H), 4.39 (t, J = 7.3 Hz, 1H, 11-H) 3.96 (t, J = 7.3 Hz, 1H, 11-H), 3.90 (d, J = 6.2, Hz, 1H, exchangeable, NH), 3.80 (s, 6H, 3,'5'-OCH_3), 3.10 (dd, J = 4.9, 14.0 Hz, 1H, 2-H), 3.02 (m, 1H, 3-H). Anal. $C_{27}H_{24}BrNO_7$) C.H.N.

EXAMPLE 40

4'-Demethyl-4ß-[4"-bromoanilinyl]-4-desoxypodophyllotoxin.

Crystals from ethyl acetate/ethanol; mp 227-230°C;$[\alpha]^{25}D$-110° (c = 0.5, $CHCl_3$); IR (KBr) 3500 (OH), 3330 (NH), 2900 (aliphatic CH), 1755 (lactone), 1605, 1590 and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30 (d, J = 8.9, Hz, 2H, 3",5"-H), 6.75 (s, 1H, 5-H), 6.53 (s, 1H, 8-H), 6.44 (d, J = 8.9 Hz, 2H, 2",6"-H), 6.32 (s, 2H, 2',6'-H), 5.98 (ABq, J = 1.3, 8.3 Hz, 2H, OCH_2O), 5.42 (s, 1H, exchangeable, 4'-OH), 4.62 (m, 2H, 4-H and 1-H), 4.36 (t, J = 8.5 Hz, 1H, 11-H), 3.95 (t, 8.5 Hz, 11-H), 3.86 (d, J = 7.8, 1H, exchangeable, NH), 3.79 (s, 6H, 3,'5'-OCH_3), 3.11 (dd, J = 4.8, 14.1 Hz, 1H, 2-H), 3.00 (m, 1H, 3-H). Anal. $C_{27}H_{24}BrNO_7$) C.H.N.

EXAMPLE 41

4'-Demethyl-4ß-[4"-iodoanilinyl]-4-desoxypodophyllotoxin.

Crystals from ethanol; mp 198-200°C(dec.);$[\alpha]^{25}D$-111° (c = 0.5, $CHCl_3$); IR (KBr) 3540 (OH), 3420 (NH), 2900 (aliphatic CH), 1770 (lactone), 1610, 1585 and 1480 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.46 (d, J = 8.8 Hz, 2H, 2",6"-H), 6.32 (s, 2H, 2',6'-H), 5.59 (ABq, J = 1.1, 8.9 Hz, 2H, OCH_2O), 5.44 (s, 1H,

exchangeable, 4'-OH), 4.62 (m, 1H, 4-H), 4.58 (d, J = 4.9 Hz, 1H, 1-H), 4.34 (t, J = 8.5 Hz, 1H, 11-H), 3.94 (m, 2H, 11-H and NH), 3.78 (s, 6H, 3',5'-OCH_3), 3.09 (dd, J = 4.9, 14.1 Hz, 1H, 2-H), 2.99 (m, 1H, 3-H). Anal. $C_{27}H_{24}BrNO_7$) C.H.N.

EXAMPLE 42 - 44

(Scheme IX)

Podophyllotoxin (500mg, 1.2 mmol) was dissolved in dry dichloromethane (10ml) and kept at 0°C. Hydrogen bromide gas was introduced into the solution for 45 min., after which time the solvent was evaporated in vacuo, anhydrous tetrahydrofuran (15 ml), anhydrous barium carbonate (474 mg, 2.4 mmol) and the appropriate hydroxyaniline (142mg, 1.3 mmol) was added. The mixture stood at room temperature overnight, and then was filtered and concentrated. The crude product was purified using column chromatography (silica gel 45 g with dichloromethane-acetone-ethyl acetate 100:5:5 as an eluant). The products (42-44) obtained in the examples had the characteristics listed below.

EXAMPLE 42

4β-[2"-hydroxyanilinyl]-4-desoxypodophyllotoxin.

Amorphous crystals from ether: mp 145-148°C; IR (KBr) 3480 (OH), 3410 (NH), 2900 (aliphatic CH), 1760 (lactone), 1580, 1475 (aromatic C=C) cm^{-1}; H NMR ($CDCl_3$) δ 6.90 (t,J = 6.6 Hz, 1H, 4"-H), 6.78 (s, 1H, 5-H), 6.65 (m, 2H, 3",6"-H), 6.53 (m, 2H,8-H, 5"-H), 6.34 (s, 2H 2',6'H), 5.96 (ABq, J = 1.0, 3.5 Hz,2H, OCH_2O), 5.02 (s, 1H, exchangeable, 2"-OH), 4.68 (m, 1H, 4-H), 4.62 (d, J = 4.9 Hz, 1H, 1-H),

4.38 (t, J = 8.6 Hz, 1H, 11-H), 4.33 (m, 1H, exchangeable, NH), 4.00 (t, J = 8.6 Hz, 1H, 11H), 3.82 (s, 3H, 4'-OCH_3), 3.76 (s, 6H, 3',5'-OCH_3), 3.25 (dd, J = 5.1, 14.0 Hz, 1H, 2-H), 3.05 (m, 1H, 3-H). MS, m/z = 505 (m+). Anal. ($C_{28}H_{27}NO_8 \cdot 3/2\ H_2O$) C.H.

EXAMPLE 43

4ß-[3"-hydroxyanilinyl]-4-Desoxypodophyllotoxin.

Amorphous powder from ether, mp 148-150°C; IR(KBr) 3370 (OH,NH), 2900 (aliphatic CH), 1760 (lactone), 1585, 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.05 (t, J = 8.0 Hz, 1H, 5"-H), 6.77 (s, 1H, 5H), 6.52 (s, 1H, 8-H), 6.32 (s, 2H, 2' 6'-H), 6.25 (dd, J = 2.2, 8.0 Hz, 1H, 4"-H), 6.14 (dd, J = 2.2, 8.0 Hz, 1H, 6"-H), 6.05 (t = 2.2 Hz, 1H, 2"-H), 5.96 (ABq, J = 1.3, 3.8 Hz, 2H OCH_2O), 4.64 (d, J = 3.9 1H, 4-H), 4.49 (d, J = 5.0, Hz, 1H, 1-H), 4.4 (t, J = 8.7 Hz, 1H, 11-H), 4.03 (t, J = 8.7 Hz, 1H, 11-H), 3.81 (s, 3H, 4'OCH_3), 3.76 (s, 6H, 3',5'-OCH_3), 3.18 (s, 6H, 3',5'-OCH_3), 3.18 (dd, J = 5.0 Hz, 14.0 Hz, 1H,2-H), 3.02 (m, 1H, 3-H); MS, m/z = 505 (m+). Anal. ($C_{28}H_{27}NO_8 \cdot H_2O$) C.H.

EXAMPLE 44

4ß-[4"-hydroxyanilinyl]-4-desoxypodophyllotoxin.

Crystals from chloroform; mp 145-150°C; IR(KBr) 3310 (OH,NH), 3010 (aromatic CH), 2900 (aliphatic CH), 1730 (latone), 1575, 1475 (aromatic C=H) cm^{-1}; ^1H NMR ($CDCl_3$) D_2O exchange) δ 6.75 (d, J = 8.3 Hz, 3H, 5-H, 3",5"-H), 6.53 (s, 1H, 8H), 6.45 (d, J = 8.3 Hz, 2H, 2",6"-H), 6.23 (s, 2H 2'6'-H), 5.95 (ABq, J = 1.0, 4.0 Hz, 2H OCH_2O), 4.60 (d, J = 4.2 Hz, 1H, 4-H), 4.57 (d, J = 4.6 Hz, 1H, 1-H), 4.38 (t, J = 6.0 Hz, 1H, 11-

H), 4.05 (t, J = 6.0 Hz, 1H, 11H) 3.83 (s, 3H, 4-OCH_3), 3.75 (s, 6H, 3',5'-OCH_3), 3.18 (dd, J = 4.6 Hz, 14.0 Hz, 1H,2-H), 3.0 (m, 1H, 3-H).

Anal. ($C_{28}H_{27}NO_8 \cdot 1/2\ H_2O$) C.H.

Isolation of Human DNA Topoisomerase II.

Human DNA topoisomerase II was isolated from peripheral blast cells of a patient with acute leukemia. The isolation procedure is described in Thurston, L., Imakura, Y., Haruna, M., Li, Z. C., Liu, S. Y., and Lee, K. H., J. Med. Chem., 31, COMPLETE (1988) and is a partial combination of the procedure described in Goto, T., Laiapia, P. and Wang, J., J. Biol. Chem., 259, 10422 (1984) and Halligan, B., Edwards, K., and Liu, L., J. Biol. Chem., 260, 2475 (1985) which are herein specifically incorporated by reference.

Preparations of Drugs.

Drugs were dissolved in Me_2SO at a concentration of 20 mM as the stock solution and diluted before use with water to the desired concentration of each drug.

DNA Topoisomerase II Assay.

The P4 unknotting reaction was a modification of the procedure described by Hseih, T., J. Biol. Chem., 258, 8413 (1985), which is herein specifically incorporated by reference.

The reaction mixture (20 μL), which contained 50 mM HEPES, pH 7.0, 50 mM KCI, 100 mM NaCl, 0.1 mM EDTA, 10 mM $MgCl_2$, 1.0 mM ATP, 50 μg/mL bovine serum albumin, 0.4 μg P4 knotted DNA, and enzyme, was incubated with or without drugs.

The reaction mixture was incubated at 37° C for 30 min and terminated by adding 5.0 µl of a stop solution (2% sodium dodecyl sulfate, 20% glycerol, 0.05% bromophenol blue). These samples were loaded onto a 1% agarose gel and electrophoresed at 55 V overnight with an electrophoresis buffer that contained 90 mM Tris-boric acid, pH 8.3, and 2.5 mM EDTA. At completion, the gel was stained in 0.5 µg/mL of ethidium bromide. Then a photograph was taken of the DNA bands visualized with fluorescence induced by a long-wavelength UV lamp. The data reported in Table 1 reflect a 100 µM drug concentration.

K-SDS Precipitation Assay for Protein-DNA Complexes.

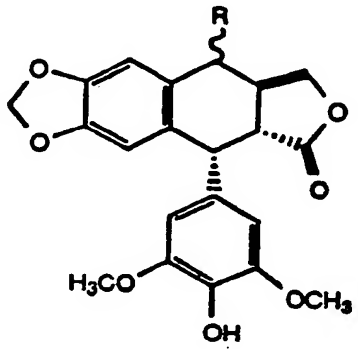
The intracellular formation of covalent topoisomerase II-DNA complexes was quantitated using the potassium SDS precipitation assay, a procedure adapted from the method described in Rowe, T.C., Chen, G. L., Hsiang, Y. H., and Liu, L., Cancer Res., 46, 2021 (1986) (hereinafter Rowe et al.), which is herein specifically incorporated by reference. KB ATCC cells were prelabeled with 0.05 mCi/ml ^{14}C-thymidine (specific activity 50.5 mCi/mmol) for 18 hr. A final concentration of $5x10^5$ cells/sample were treated with 10 µM of the drugs at 37° C for 1 hr and proceeded according to the procedure described by Rowe et al. to detect the protein-linked DNA levels.

It will be apparent to those skilled in the art that various modifications and variations can be made in the processes and products of the present invention. Thus, it is intended that the present invention cover the

modifications and variations of this invention provided that they come within the scope of the appended claims and their equivalents.

TABLE I

BIOLOGICAL EVALUATION OF 4'-DEMETHYL-4-ALKYLAMINO-PODOPHYLLOTOXIN ANALOGUES



COMPOUND	R	CYTO-TOXICITY[a] ED_{50}KB(μg/ml)	DNA TOPO-ISOMERASE II ACTIVITY % INHIBITION[b]	CELLULAR PROTEIN-DNA COMPLEX FORMATION[c]%
Etoposide		0.20	+++	100.0
Example:				
1	β-OH	0.34	++	42.2
2	α-OH	0.045	+	3.3
3	β-NH_2	1.0	++++	36.4
4	α-NH_2	0.42	+	8.0
5	β-$NHCH_2CH_2OH$	1.6	++++	121.4
6	α-$NHCH_2CH_2OH$	710.0	-	0.0
7	β-$NHCH_2CH_2CH_3$	<0.4	++	69.7
8	β-$NHCH_2CH_2OCH_3$	>4.0	+++	110.8
9	β-$NHCH_2CH=CH_2$	3.4	+++	84.1
10	β-$NHCH_2CH(OH)CH_3$	3.6	++++	167.2
11	β-$NHCH(CH_3)CH_2OH$	2.3	++++	161.7
12	β-$NHCH_2CH_2CH_2OH$	4.0	++	89.2
13	β-$OCH_2CH_2NH_2$	0.1	++++	300.0
14	β-OCH_2CH_2OH	0.7	++	50.0

[a] ED_{50} is the concentration of drug which affords 50% reduction in cell number after 3 days incubation.

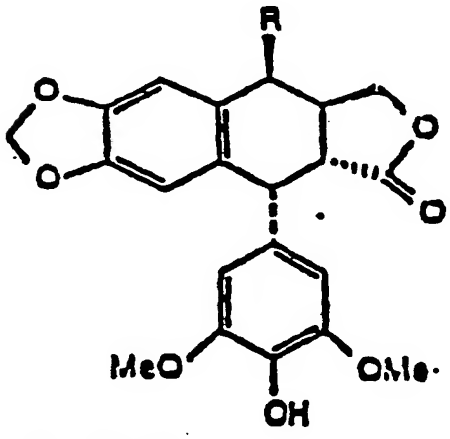
[b] +, ++, +++, ++++ and - denote for 25%, 50%, 75%, >75%, and 0% inhibition.

[c] Relative activities of cellular protein-DNA complex formation in KB ATCC tissue culture cells measured at 10 μM drug concentration as compared the complex formed by 10 μM of etoposide.

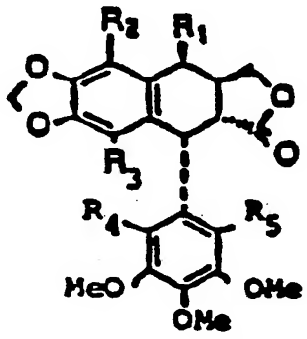
TABLE II

COMPOUNDS WITH POTENT TOPOISOMERASE II INHIBITORY ACTIVITY

COMPOUNDS	DNA TOPOISOMERASE II INHIBITORY ACTIVITY[a] (RELATIVE POTENCY)
Etoposide	1.0
15	2.0 - 6.0
16	8.0
17	2.0
18	2.0



15 R=Cl



16 $R_1=R_2=R_3=R_5=Br, R_4=H$

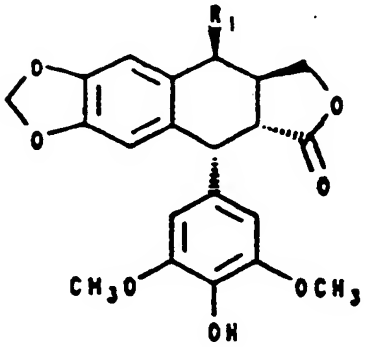
17 $R_1=OH$, $R_2=R_4=R_5=Br, R_3=H$

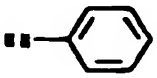
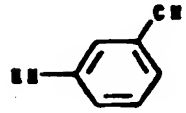
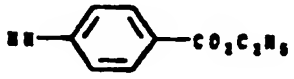
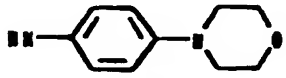
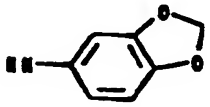
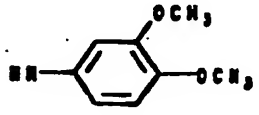
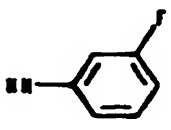
18 $R_1=OH$, $R_2=R_3=R_4=R_5=Br$

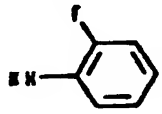
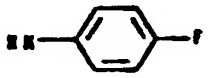
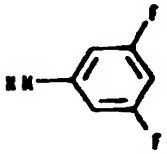
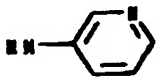
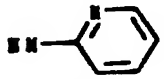
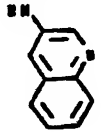
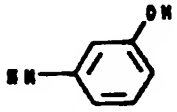
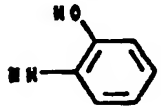
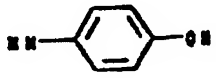
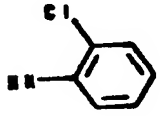
[a] Several different concentrations of tested compounds were employed for the determination of their potency. The relative potency with respect to etoposide as expressed in Table I was the relative concentration of compounds tested to achieve the same degree inhibition by etoposide in the range of 25 to 400 μM.

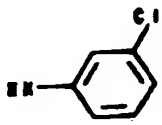
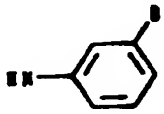
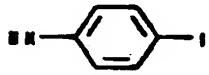
Table III

Biological Evaluation of 4-Demethyl-4β(arylamino)-4-desoxy Podopyllotoxin



Compound	R_1	DNA Topoisomerase II Activity % Inhibition	Cellular Protein-DNA Complex Formation	Cytotoxicity ID_{50} K_a (uM)
Etoposide		+++	100	0.2
19		+++	243	0.71
20		++++	211	0.64
21		+++	137	0.69
22		+++	207	<0.10
23		+++	140	0.66
24		++++	164	<1.0
25		++	180	<1.0
26		+++	158	0.23

Compound	R_1	DNA Topoisomerase II Activity % Inhibition	Cellular Protein-DNA Complex Formation	Cytotoxicity ID_{50} K_8 (uM)
27		++	121	0.25
28		++++	213	0.24
29		++	115	1.08
30		+++	148	0.24
31		++	97	0.71
32		++	123	<1.0
33		++++	290	0.45
34		++++	151	4.54
35		+++	211	2.26
36		+	32	2.34

Compound	R_1	DNA Topoisomerase II Activity % Inhibition[b]	Cellular Protein-DNA Complex Formation[c]	Cytotoxicity ID_{50} K_s (uM)[a]
37		++	51	2.29
38		+++	99	0.22
39		+	62	2.36
40		++	179	0.22
41		+	64	0.34

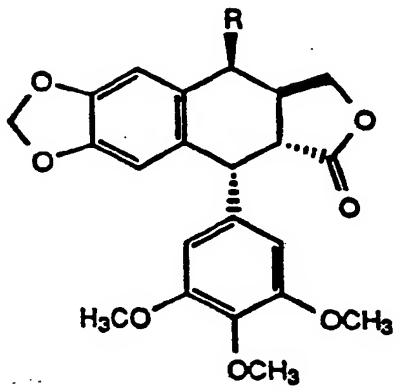
[a] ID_{50} is the concentration of drug which affords 50% reduction in cell number after 3 days incubation.

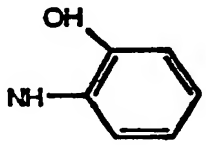
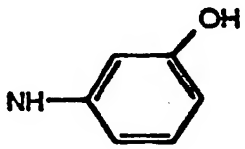
[b] Activities of cellular protein-DNA complex formation in KB ATCC tissue culture cells relative to Etoposide.

[c] +,++,+++,++++ denote 0-24%,25-49%,50-74%, and ≥75% inhibition respectively.

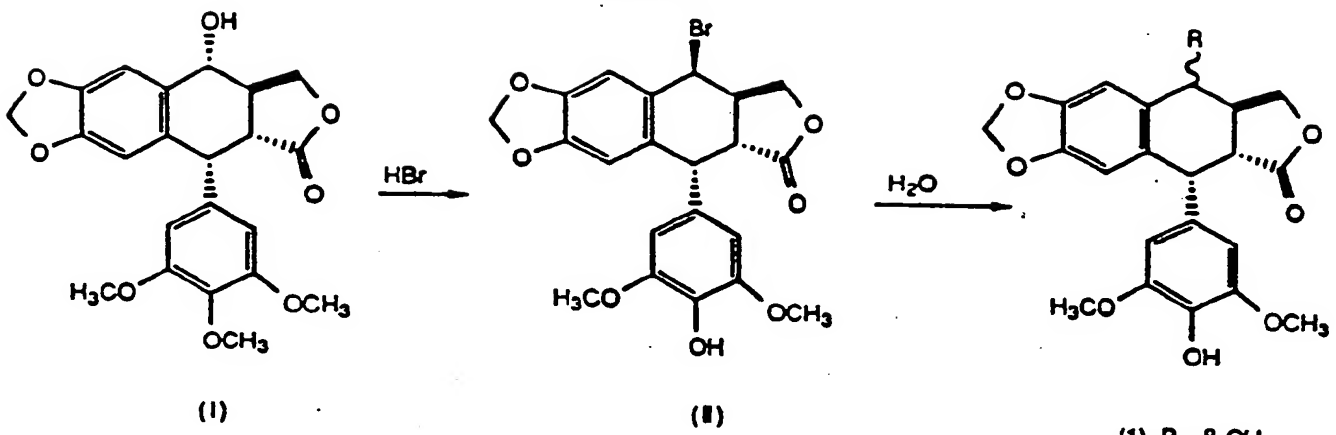
Table IV

Biological Evaluation of 4β-(arylamino)-4-desoxypodophyllotoxins



Compound	R	DNA Topoisomerase II Activity % Inhibition	Cellular protein-DNA Complex Formation	Cytotoxicity ID_{50} KB (μM)
Etoposide		+++	100	0.2
42		+	6	4.11
43		+	37	0.31
44		+	21	0.31

Scheme I

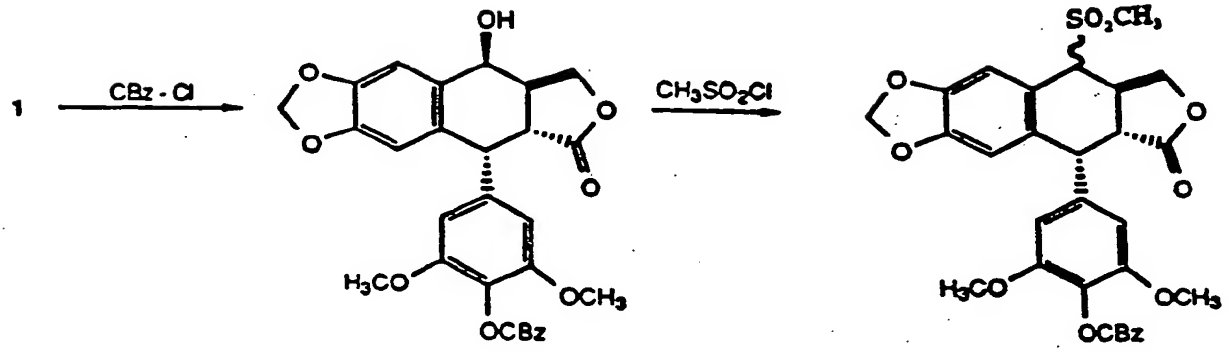


(I) (II)

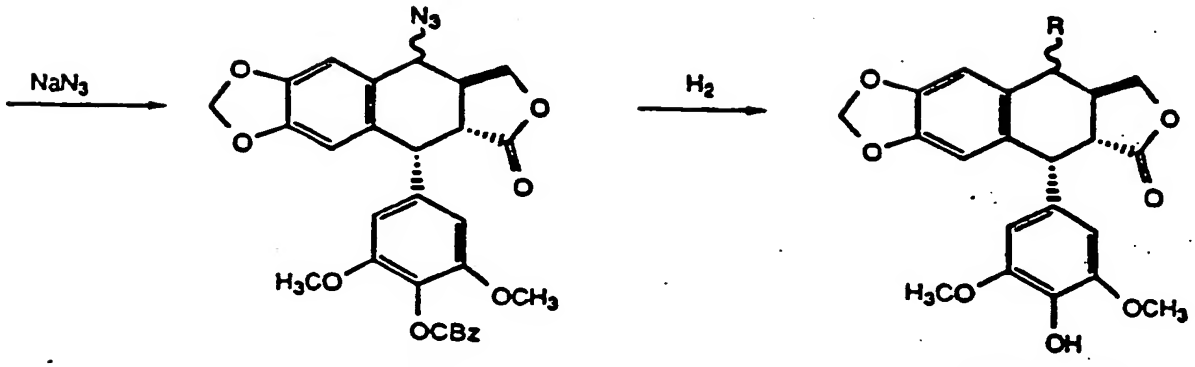
(1) R = β-OH

(2) R = α-OH

Scheme II



(IV) (V)

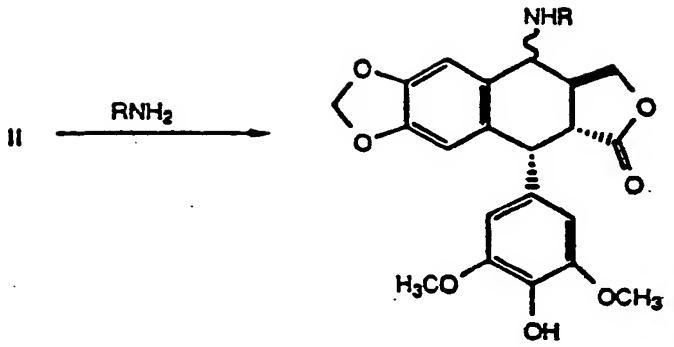


(VI)

(3) R = β-NH2

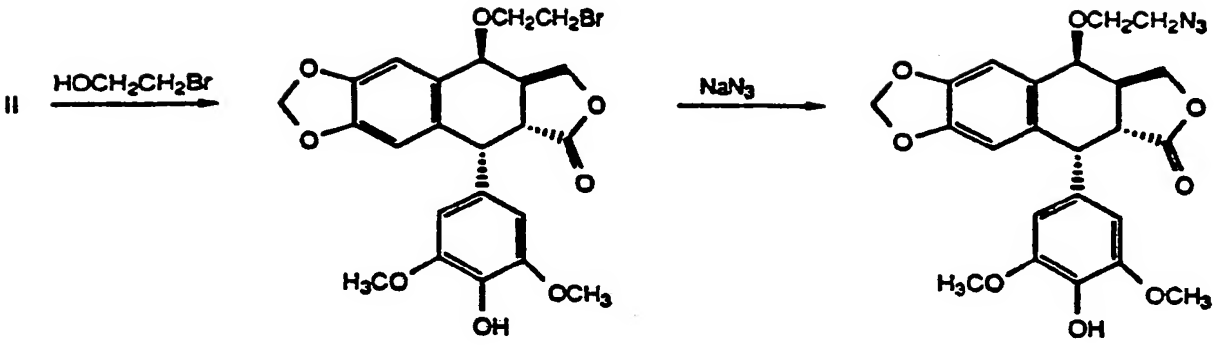
(4) R = α-NH2

Scheme III

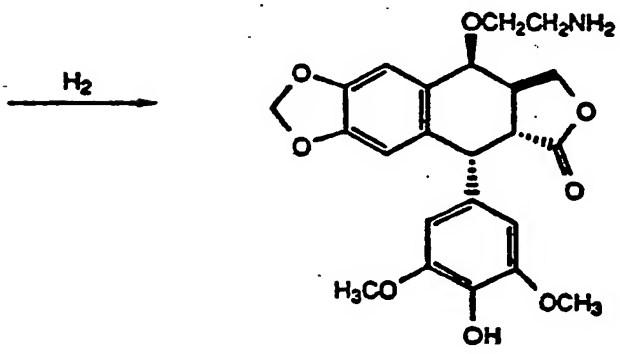


(5 - 12)

Scheme IV

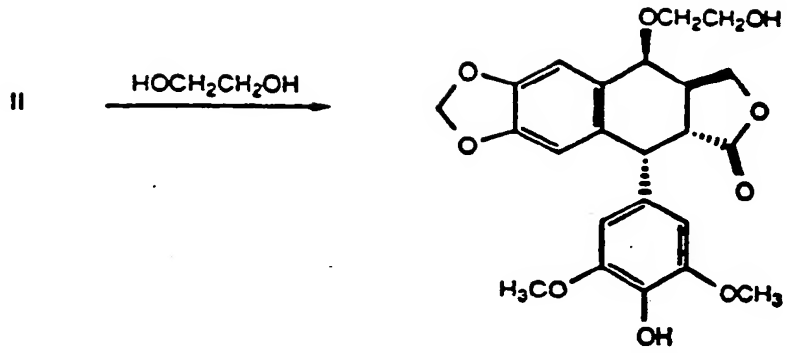


(V) (VI)



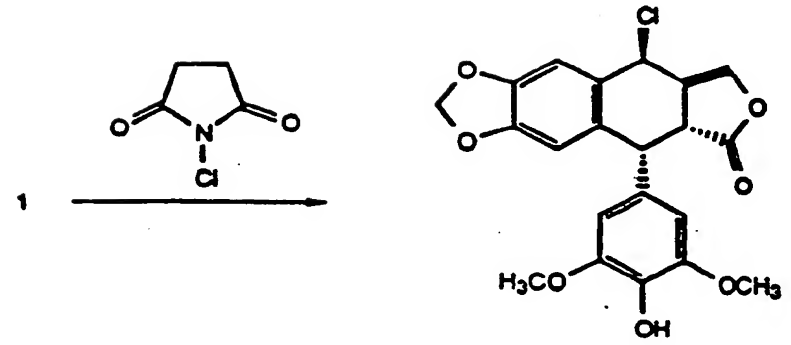
(13)

Scheme V



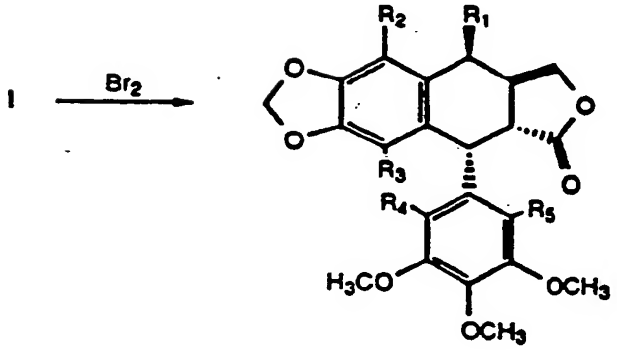
(14)

Scheme VI



(15)

Scheme VII

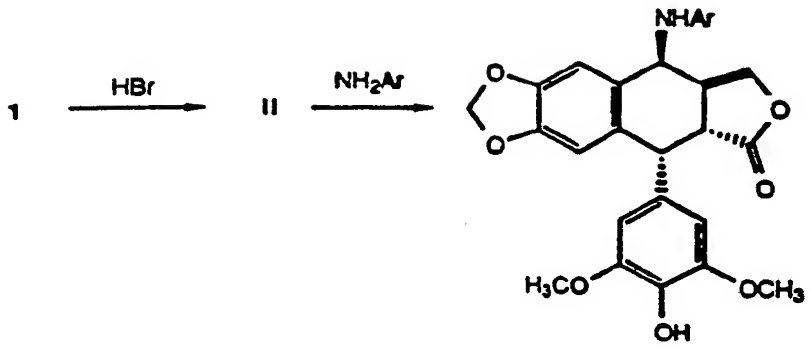


(16) R_1=R_2=R_3=R_5=Br, R_4=H

(17) R_1=OH, R_2=R_4=R_5=Br, R_3=H

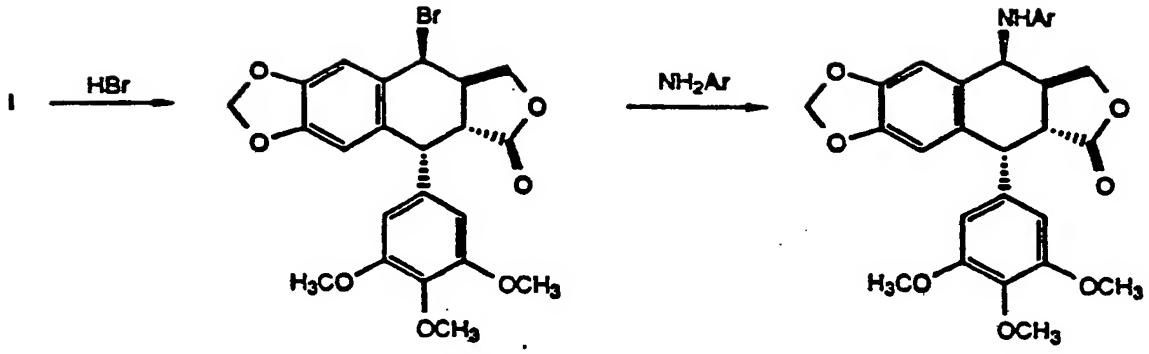
(18) R_1=OH, R_2=R_3=R_4=R_5=Br

Scheme VIII



(19 - 41)

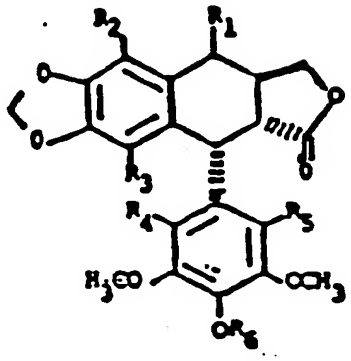
Scheme IX



(42 - 44)

WHAT IS CLAIMED IS:

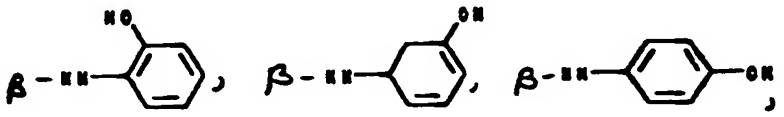
1. A compound having the formula:



where:

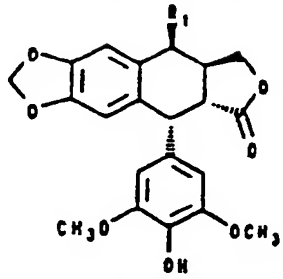
R_1 is β-$OCH_2CH_2NH_2$, β-$NHCH(CH_3)CH_2OH$, β-$NHCH_2CH(CH_3)OH$, β-Cl, β-Br, β-OH, α-OH, β-NH_2, α-NH_2, β-$NHCH_2CH_2OH$, α-$NHCH_2CH_2OH$, β-$NHCH_2CH_2CH_3$, β-$NHCH_2CH_2OCH_3$, β-$NHCH_2CH{=}CH_2$, β-$NHCH_2CH(OH)CH_3$, β-$NHCH_2CH_2CH_2OH$; or β-OCH_2CH_2OH;

wherein R_1 is



R_2 is H, or Br;
R_3 is H, or Br;
R_4 is H, or Br;
R_5 is H, or Br; and
R_6 is H, or $-CH_3$.

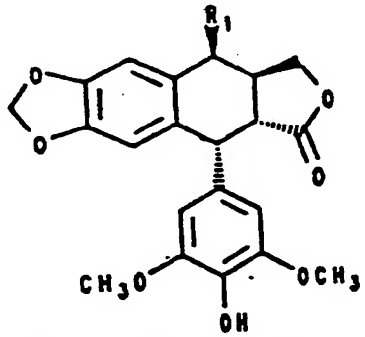
2. A compound having the formula:



where:

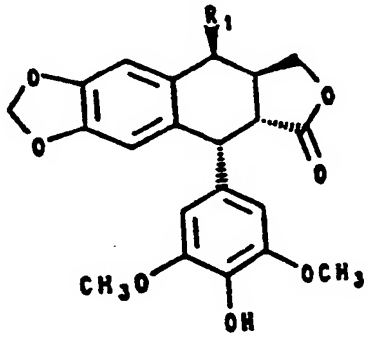
R_1 is $-NHCH_2CH_2OH$, $-NHCH_2CH_2OCH_3$, $-NHCH_2CH(OH)CH_3$, $NHCH(CH_3)CH_2OH$, or Cl.

3. A compound having the formula:

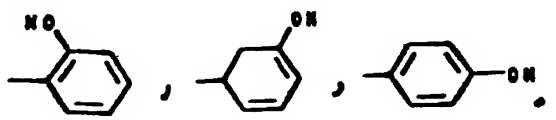


where R_1 is $-OCH_2CH_2NH_2$ or $-NHCH_2CH_2OH$

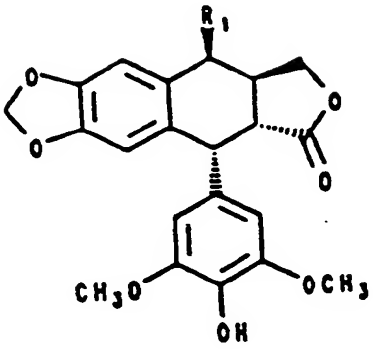
4. A compound having the formula:



where R_1 is $-NHCCH_3HCH_2OH$, $-NHCH_2CCH_3HOH$, or NHR_4, wherein R_4 is

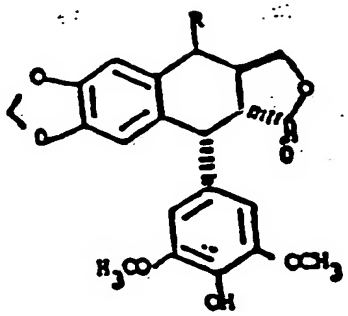


5. A compound having the formula:



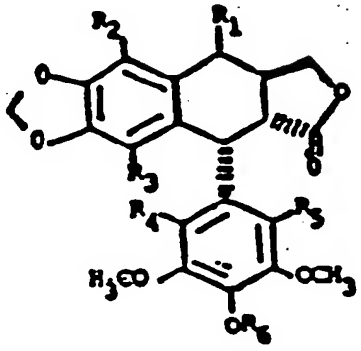
where R_1 is $-NHCH_2CH_2OH$.

6. A compound having the formula:



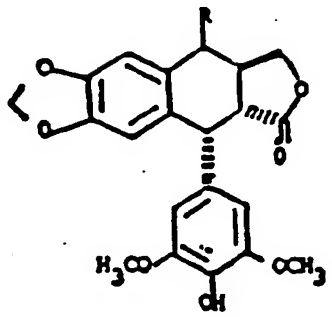
where R is Cl or Br.

7. A compound having the formula:



where R_1 is Cl, Br, or OH;
R_2 is H, or Br;
R_3 is H, or Br;
R_4 is H, or Br;
R_5 is H, or Br; and
R_6 is H, or $-CH_3$.

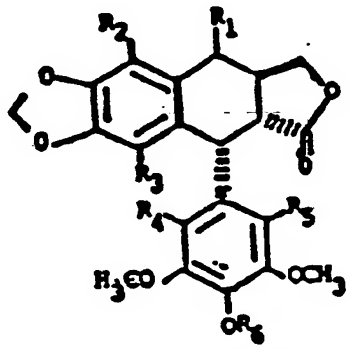
8. A compound having the formula:



where R is β-OH, β-NH_2, β-$NHCH_2CH_2OH$, β-$NHCH_2CH_2CH_3$, β-$NHCH_2CH_2OCH_3$, β-$NHCH_2CH{=}CH_2$, β-$NHCH_2CH(OH)CH_3$, β-$NHCH_2CH_2CH_2OH$, β-OCH_2CH_2OH or β-$OCH_2CH_2NH_2$.

9. A process for treating tumors in humans and lower animals by administering a safe and effective amount of a compound according to claim 1.

10. A pharmaceutical composition comprising a compound having antitumor activity of the formula:

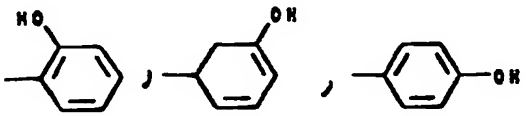


where:

R_1 is β-$OCH_2CH_2NH_2$, β-$NHCCH_3HCH_2OH$, β-$NHCH_2CCH_3HOH$, β-NHR_6, β-Cl, β-Br, β-OH, α-OH, β-

NH_2, α-NH_2, β-$NHCH_2CH_2OH$, α-$NHCH_2CH_2OH$, β-$NHCH_2CH_2CH_3$, β-$NHCH_2CH_2OCH_3$, β-$NHCH_2CH{=}CH_2$, β-$NHCH_2CH(OH)CH_3$, β-$NHCH_2CH_2CH_2OH$, β-OCH_2CH_2OH;

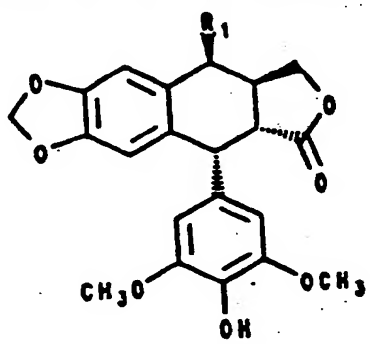
wherein R_4 is



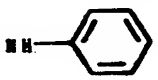
R_2 is H, or Br;
R_3 is H, or Br;
R_4 is H, or Br;
R_5 is H, or Br; and
R_6 is H, or $-CH_3$.

11. A compound according to claim 1 exhibiting antitumor activity.

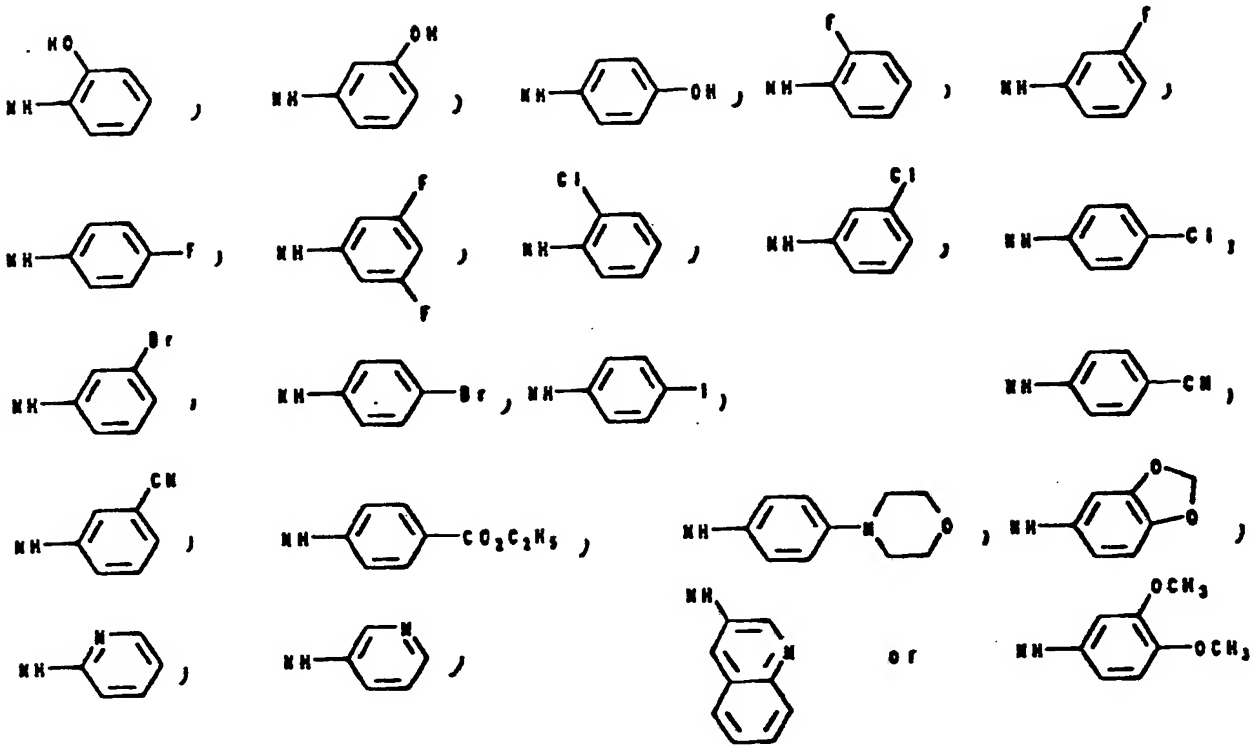
12. A compound of the formula:



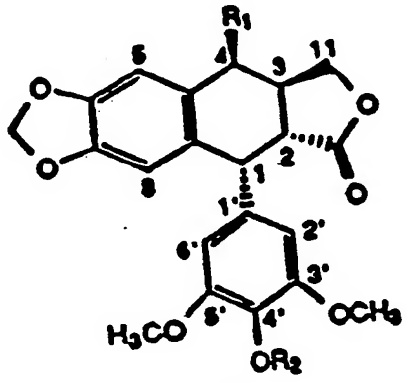
where R_1 is a substituted or unsubstituted arylamine.

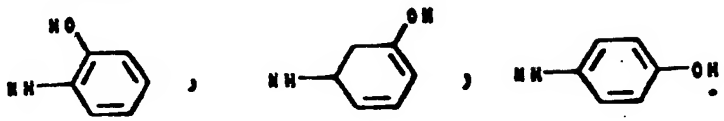
13. A compound according to claim 12 where R_1 is  .

14. A compound according to Claim 12 where R_1 is selected from:



15. A compound of the formula:



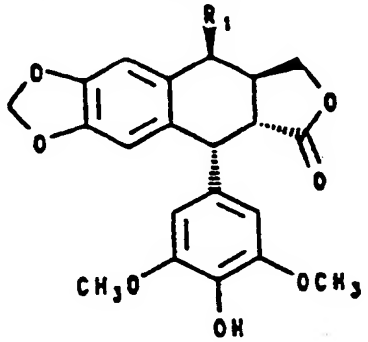
Where R_1 is  and R_2 is CH_3.

16. A process for treating tumors in humans and lower animals by administering a safe and

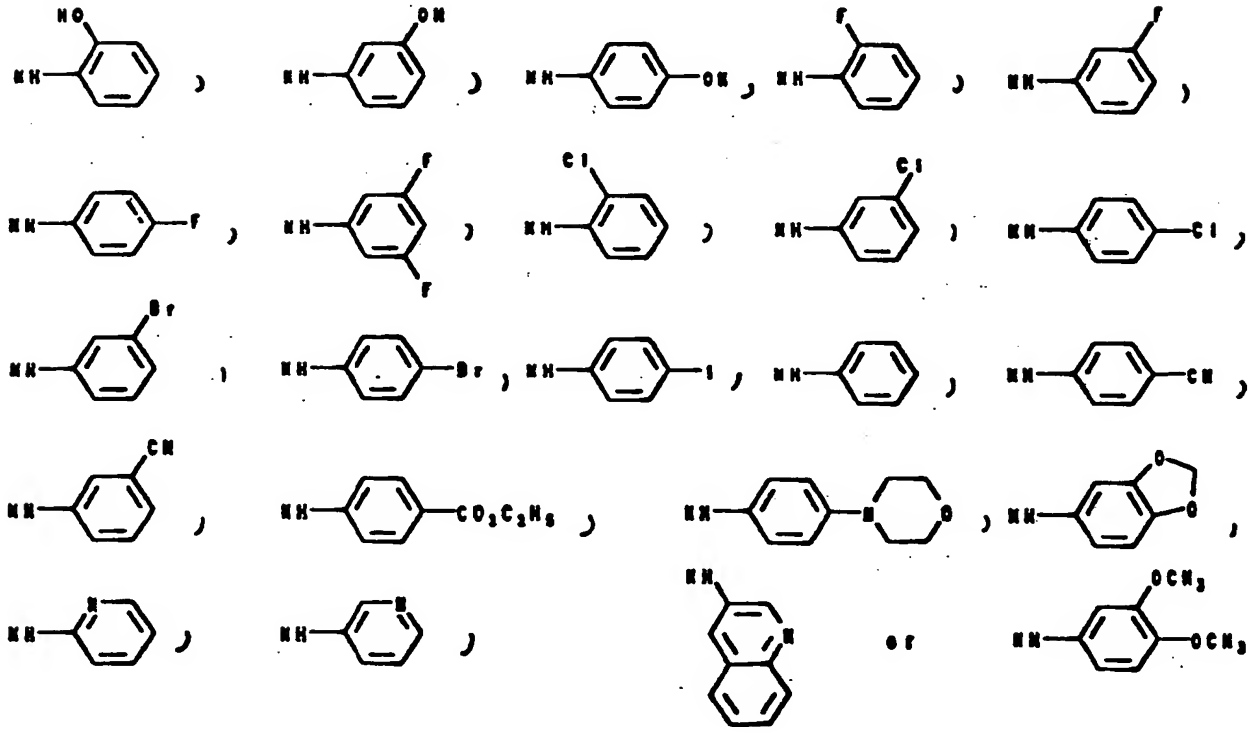
effective amount of a compound according to claim 12.

17. A compound according to claim 12 exhibiting antitumor activity.

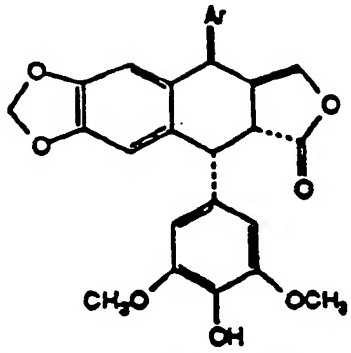
18. A pharmaceutical composition comprising a compound having the formula:



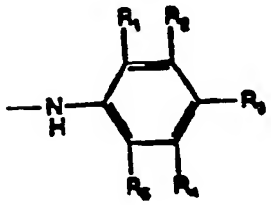
where R is selected from:



19. A compound of the formula:

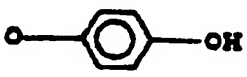


wherein Ar is an arylamine as in the formula:



wherein R_1 is H, OH, F, Cl, Br, CO_2CH_3, $CO_2C_2H_5$, CN, NO_2, NH_2, $N(CH_3)_2$, OCH_3, CH_2OH, CH_3, CF_3, CH_2CH_2OH, $COCH_3$, CH_2NH_2;

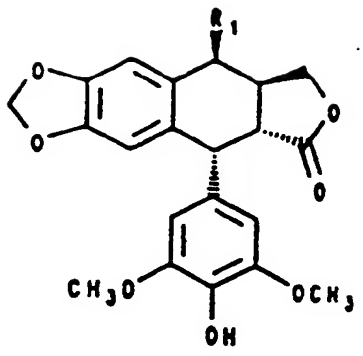
R_2 is H, OH, F, Cl, Br, CO_2CH_3, $CO_2C_2H_5$, CN, NO_2, NH_2, $N(CH_3)_2$, OCH_3, CH_2OH, CH_3, CF_3, CH_2CH_2OH, $COCH_3$, CH_2NH_2, $CHOHCH_3$, SCH_3, CH_3, CO_2CH_3;

R_3 is H, OH, F, Cl, Br, I, CO_2CH_3, $CO_2C_2H_5$, CN, NO_2, NH_2, $N(CH_3)_2$, OCH_3, CH_2OH, CH_3, CF_3, CH_2CH_2OH, $COCH_3$, CH_2NH_2,  ,  ,  , $N(CH_2CH_2OH)_2$, CH_3;

R_4 is H, F, Cl, OH, OCH_3, CO_2CH_3, $CO_2C_2H_5$, CH_3, CF_3, NO_2, NH_2, Cl;

R_5 is H, F, Cl, CH_3, CF_3, OH, OCH_3, NO_2;
R_3 and R_4 are OCH_2O or OCH_2CH_2O.

20. A compound of the formula:



wherein R_1 is a flat aromatic ring system, and said ring system contains a heteroatom or is substituted with an electron donating group at the 3 or 4 position on the ring.

21. A compound according to Claim 20 wherein the electron donating substituent at the 3 or 4 position is oxygen.

22. A compound according to Claim 20 wherein the flat aromatic ring system is pyridine.

23. A process for treating tumors in humans and lower animals by administering a safe and effective amount of a compound according to claim 18.

24. A process for treating tumors in humans and lower animals by administering a safe and effective amount of a compound according to claim 19.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/00842

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 31/34; C07D; 307/83, 311/44; A61K 31/36.44,47,555; C07D 215/38, 317/70, 319/12, 401/02,413/10. US CL. 514/463,468; 549/298;514/232.5,313,338,467;544/148;546/174,270; 549/358,432

II FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
U.S.	514/463,468; 549/298; 514/232.5, 313,338,467; 544/148; 546/174,270;549/358.432

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X	US, A, 2,977,359 (RUTSCHMANN), 28 March 1961 See the entire document.	1,9,10
X	US, A, 2,984,674 (RUTSCHMANN), 16 May 1961 See the entire document.	1,9,10
Y	US, A, 3,524,844 (KELLER-JUSLEN et al).18 August 1970 See the entire document.	1,9,10
X	US, A, 4,122,092 (KENDE et al), 24 October 1978 See the entire document.	1,9,10
X	US, A, 4,567,253 (DURST et al), 28 January 1986 See the entire document.	1,9,10
X	US, A, 4,788,216 (LEANER et al), 29 November 1988 See the entire document.	1,9,10
X	JP, A, 63-10789 (NIPPON KAYAKU KK), 18 January 1988 See the abstract.	1,9,10
X	JP, A, 63-23884 (NIPPON KAYAKU KK), 30 January 1988 See the abstract	1,9,10

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
01 MAY 1990	04 JUN 1990
International Searching Authority	**Signature of Authorized Officer**
ISA/US	BA K. TRINH

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y P	JP, A, 1-93589 (ZH BISEIBUTSU KAGAKU KEN), 12 April 1989. See the abstract.	1,9,10
X	N, Chemical Abstracts, Vol. 105, entry 60444U, 1986, (THURSTON et al).	1,9,10
X	N, Helvetica Climica Acta, Vol. 52, No. 4, 1969, Article No. 106, pp 944-947, (KUHN et al)	1,9,10
X	JP,A, 1-117885 (DSIICHI SEIYAKU KK), 10 MAY, 1989. SEE THE ABSTRACT	1,9,10
A	US, A, 4,144,336 (BOLTZE ET AL.), 13 March 1979. SEE THE ENTIRE DOCUMENT.	14, 18, 19, 23, 24
A	US, A, 4,808,592 (NELSON ET AL.), 28 February 1989. SEE THE ENTIRE DOCUMENT.	14, 18, 19,

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1 ☐ Claim numbers . because they relate to subject matter[1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out[1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

SEE ATTACHMENT

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Continuation of VI: Unity of Invention is Lacking

I. Claims 1 to 11, drawn to generic compounds and method of use the compounds containing acyclic derivatives, classifiable as 549/298, 514/467.

II. Claims 1,4,10,12 to 21,23 and 24, drawn to generic compounds, composition and method of use the compounds containing aromatic derivatives excluding the heterocyclic substituents, classifiable as 549/298, 514/232.5.

III. Claims 12,14,17 to 20,23 and 24 drawn to generic compound, composition and method of use the compound containing morpholine group, classifiable as 544/148.

IV. Claims 12,14,17 to 20,23 and 24, drawn to generic compound, composition and method of use the compound containing 5-membered dioxolene group, classifiable as 549/432.

V. Claims 12,17,19,20 and 24, drawn to generic compound and method of use the compound and method of use the compound containing 6-membered dioxolene classifiable as 549/358.

VI. Claims 12,17,18,20,22 and 23, drawn to generic compound, composition and method of use the compound containing pyridine group, classifiable as 546/270, 514/338.

VII. Claims 12,14,17,18, 20 and 23, drawn to generic compound, composition and method of use the compound containing quinoline group, classifiable as 546/174, 514/313.

Under PCT regulations, applicants are entitled to have a product, a composition and a process of using searched, based on the filing fee.

The various claimed inventions lack unity under PCT Rule 13 since the different substituents are distinct and capable of supporting different patents. A search for one species are not required for another species. In addition, a reference of one invention can not render the other prima facie obvious over the art in the absence of ancillary art, and one would not suggest another to a skilled artisan.